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The Journal of Laboratory and Clinical Medicine

VOL. 24 OCTOBER, 1938 NO. 1

CLINICAL AND EXPERIMENTAL

THE DIFFERENTIAL SERUM VANADATE SEDIMENTATION REACTION*

H. B. HUNT, M.B., M.R.C.S., AND D. L. WOODHOUSE, PH.D., A.I.C.,
BIRMINGHAM, ENGLAND

OUR knowledge concerning the physicochemical colloidal protein system existing in blood serum is very recent and imperfect. From clinical and pathologic standpoints some appreciation of its complexity and significance is now being afforded by researches, especially in conditions where normal equilibria appear to be definitely modified. Contributions from these aspects have been made by Coke (1937) who has applied the sodium vanadate reagent, which was introduced by Bendien (1931) as the precipitant in his diagnostic test for malignancy, employing the three-phase method of serum flocculation evolved by Cronin Lowe[4] (1933) as a means of scrutinizing the colloidal system of the serum.

One of the present authors (D. L. W.) has extensively employed the modified vanadate sedimentation reaction with a view to estimating its value as a diagnostic test for carcinoma (Jones and Woodhouse, 1936) and, although results showed that it can not be regarded as a specific test for cancer, these and subsequent studies confirm the suggestion of Cronin Lowe[6] (1933-34) and others, that when properly interpreted it affords useful information for prognosis and for control of therapeutic measures in malignant disease.

A similar conclusion is drawn by Coke[2] (1937) concerning the application of the modified vanadate reaction to arthritis and to asthma, in which he claims it to be of great practical value. To quote this investigator: "It provides (1)

*From the Asthma Clinic and the Cancer Research Laboratory, General Hospital, Birmingham.

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a reading comparable in every way to the erythrocyte sedimentation rate but . . . which is a truer indication of the actual clinical state. (2) Evidence of the state of the infectious processes present and the patient's response to these. (3) Evidence as to the 'general toxicity' and 'general resistance' of the patient. (4) The possibility of watching the progress of cases by serial examination and, in certain cases, controlling the therapeutic methods exhibited."

Since one of us (H. B. H.) was particularly interested in asthma, it was deemed of value in view of these assertions to observe the vanadate sedimentation values in asthmatics and to compare with the red blood sedimentation rates

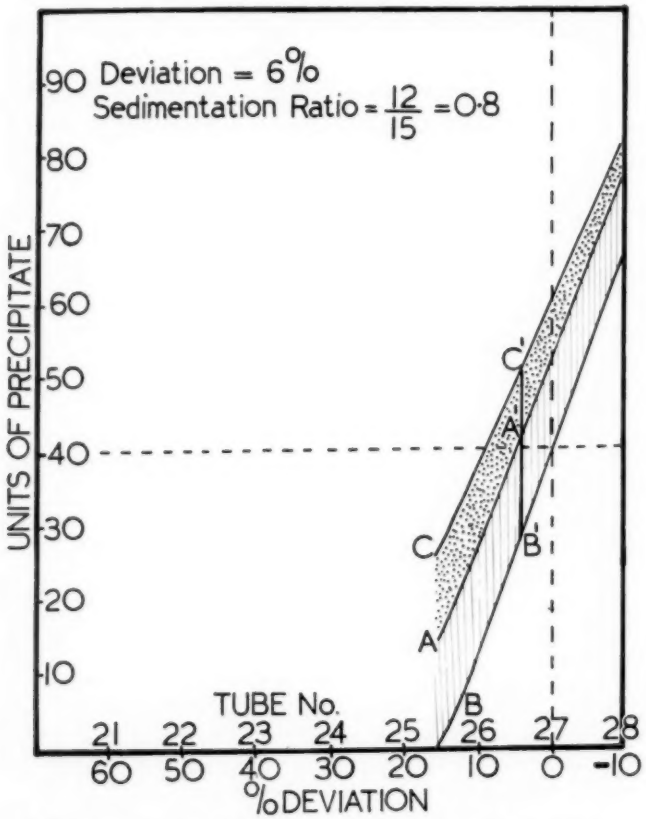

Chart 1.—Typical chart with serum from a healthy person.

and the eosinophile counts. The results of the vanadate tests, it was believed, would be of interest for comparison with those obtained from patients suffering from malignancy and from patients with pathologic but nonmalignant conditions.

Method and Rationale of Tests.—A full account of the method for estimating the differential vanadate sedimentation is given in great detail by Cronin Lowe[4] and by Coke[2] with comprehensive discussion of the physiologic, biochemical, and technical factors involved. The technique is also described in the communication by one of us previously cited (Jones and Woodhouse, 1936).

In view of this, only the following short summary is included in this report, with a brief explanation of the inferences as deduced by the elaborators.

For each serum three series of precipitation tests, A, B, and C, are set out, using for each series ten portions of 0.2 c.c., of clear, nonhemolyzed serum, which has been diluted with an equal volume of distilled water; 1 c.c. of the ten vanadate reagents (containing 21, 22, 23, to 30 c.c. of N/10 sodium vanadate, each made up to a total volume of 200 c.c. with N/10 acetic acid) is added to the tubes in order. These are numbered in accordance with the number of cubic centimeters of N/10 sodium vanadate in 200 c.c. of the reagent, viz., 21, 22, 23, etc.

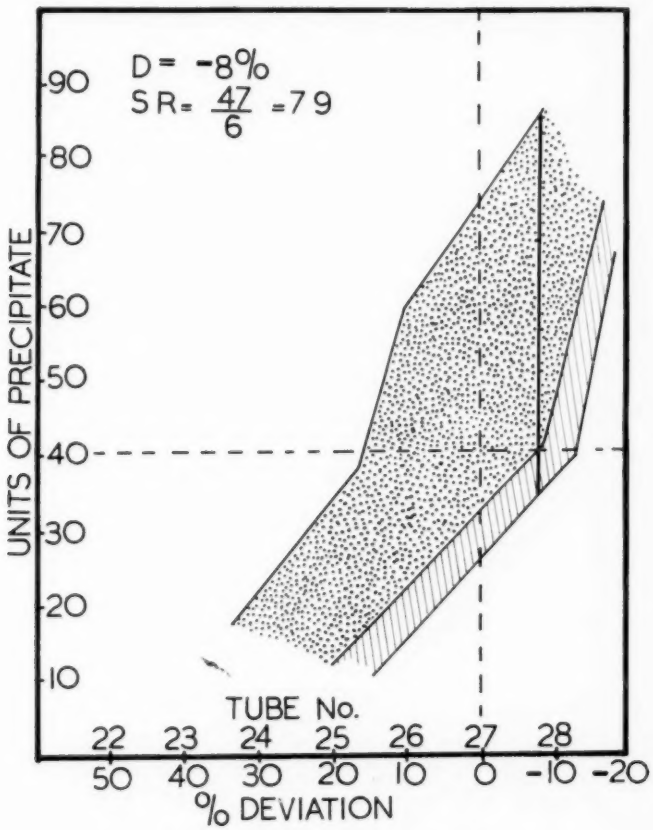

Chart 2.—Asthma "Type I" chart.

The A series is set up by using the diluted serum directly, the B series employs the same serum heated for thirty minutes at 56° C., and for the C series the serum has been treated by extraction with ether. The precipitation with the A series for healthy sera becomes definite in tubes 26 and 27, and increases with the concentration and pH of the vanadate reagent added. In pathologic cases the precipitation zone is shifted to the left or more rarely to the right. In the B series the amount of precipitation for any specified reagent is usually distinctly less than in the corresponding A series and is definitely greater in the C series.

In assessing the results for any individual test, three factors must be considered.

1. *The Differential Sedimentation Ratio.*—After estimating the amount of precipitate produced in the various tubes in suitable units, by means of the interferometer or other accurate method, the graph of the degree of flocculation is drawn for each of the three series (see charts). The sedimentation ratio may then be calculated for the specimen at any desired point. This is the difference in flocculation at that point between the A and C series compared with the difference in flocculation between the A and B series as expressed by the ratio $\frac{C - A}{A - B}$.

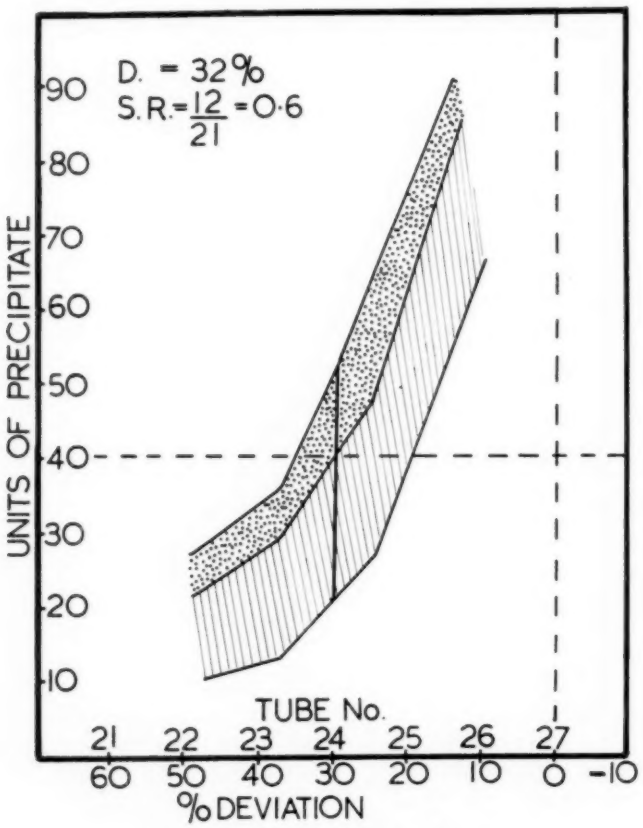

Chart 3.—Asthma "Type II" chart.

Since the most sensitive part of the precipitation zone was found to lie in the position where the precipitate from the untreated serum registered 40 units on the Zeiss interferometer when dissolved under standard conditions, the ratio is taken at this position for comparing sera (i.e., $\frac{C' - A'}{A' - B'}$, Chart 1). For normal sera this occurs in the majority of cases at tube 27.

2. *Sedimentation Deviation.*—An alteration from the "normal" so that it occurs at tube 26 (see charts) is designated a sedimentation deviation of 10 per cent, if at tube 25, 20 per cent deviation, and if at tube 28, –10 per cent deviation; i.e., one tube represents 10 per cent. The deviation may be sometimes as much as +100 per cent or –25 per cent in pathologic cases.

According to the original Bendien hypothesis, a marked positive deviation indicates malignancy, but large deviations were found to be exhibited in many pathologic conditions.

Cronin Lowe[4] suggested that the ratio $\frac{C - A}{A - B}$ at the 40 unit precipitation point was a more reliable index. This is the basis of his modified vanadate reaction, the ratio being greater than unity with malignant sera. As previously mentioned, this has been found to be not entirely reliable, but the fact has been confirmed that the ratio often becomes more normal in type when the cancer is

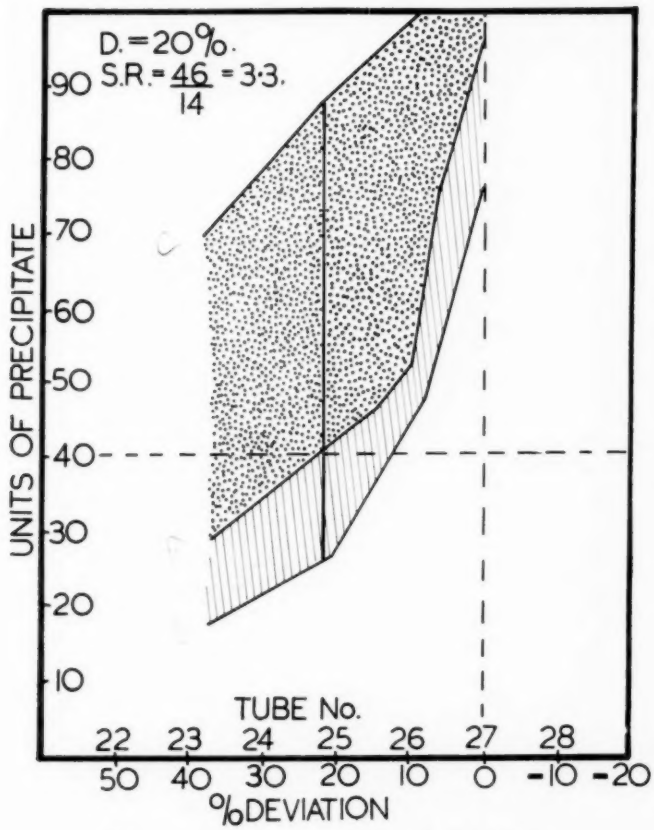

Chart 4.—Malignant case (No. 562, Table V).

responding satisfactorily to therapeutic measures. Coke believes that the whole picture expressed by the three curves (see Charts 1 to 7) should be studied in considering any case.

3. *Dotted and Striped Fields.*—The area between the A and C curves (shown by dots and called the dotted field) is believed to indicate the degree of intoxication, and the area between the A and B curves (shown by stripes and termed the striped field) to be a measure of the resources of the patient in combating the infection.

Coke also claims that by means of the differential sedimentation reaction, it is possible to divide asthmatics into three groups which correspond to the

TABLE I

CLINICAL FEATURES AND LABORATORY FINDINGS

LAB. NO.	AGE	SEX	FAMILY HISTORY	OTHER ALLERGIC DISEASE	SEVERITY OF ASTHMA	CHRONIC BRONCHITIS	TREATMENT	EOSINOPHILE COUNT PER CENT	ERYTHROCYTE SEDIMENTATION RATE IN MM.	PERCENTAGE DEVIATION	SEDIMENTATION RATIO
A											
864	14	M	Pos.	H.F.	Moderate		Nil	7.5	28	3	9.6/12.0 = 0.8
867	25	F	Pos.	H.F.	Severe	Present	Nil	3.5	3	2	4.0/10.8 = 0.4
876	13	M	Pos.	R.	Moderate	Present	Nil	8.0	25	-4	10.0/13.0 = 0.8
877	35	F	Neg.	R.	Mild	Present	Nil	7.0	6	-4	11.0/14.0 = 0.76
880	26	M	Pos.	H.F.	Moderate		Nil	3.0	2	-2	9.0/14.0 = 0.6
883	40	F	Neg.		Severe	Present	Peptone	5.0	13	2	8.0/14.0 = 0.6
884	27	F	Pos.	R.U.	Moderate		Nil	5.0	7	-2	6.0/ 8.8 = 0.7
887	20	M	Pos.	H.F., R.	Severe		Nil	8.5	5	-2	10.0/15.0 = 0.7
899	37	F	Neg.	H.F.	Moderate	Present	Pollen vaccine	0.0	4	0	12.0/16.0 = 0.8
906	32	F	Pos.	H.F.	Moderate		Nil	4.0	10	8	2.8/16.0 = 0.25
912	67	F	Neg.	R.	Moderate	Present	Bact. vaccine	1.0	5	0	8.0/10.4 = 0.8
926	38	M	Neg.	R.	Severe	Present	Peptone		3	3	6.0/12.0 = 0.5
B											
861	56	F	Pos.	H.F., R.	Moderate		Nil	1.5	11	0	32.0/ 4.4 = 7.3
901	31	F	Neg.		Mild		Torantil	6.0	9	-2	10.0/ 6.4 = 1.5
902	50	F	Pos.		Moderate		Bact. vaccine	8.0	8	0	18.8/ 6.4 = 3.0
907	14	M	Neg.	R.	Mild		Nil	6.0	3	-3	16.8/ 6.8 = 2.5
911	28	F	Pos.	H.F.	Severe		Peptone	3.5	4	6	10.0/ 6.4 = 1.7
925	37	M	Neg.	H.F.	Severe		Peptone		6	4	10.0/ 9.0 = 1.1

H. F. = Hay fever. R. = Rhinorrhea. U. = Urticaria.

TABLE II

CLINICAL FEATURES AND LABORATORY FINDINGS

LAB. NO.	AGE	SEX	FAMILY HISTORY	OTHER ALLERGIC DISEASE	SEVERITY OF ASTHMA	CHRONIC BRONCHITIS	TREATMENT	EOSINOPHILE COUNT PER CENT	ERYTHROCYTE SEDIMENTATION RATE IN MM.	PERCENTAGE DEVIATION	SEDIMENTATION RATIO
A											
836	40	F	Pos.	H.F.	Moderate		Nil	4.0	2	32	3.0/12.0 = 0.25
837	24	F	Neg.	Ecz.	Mild		Nil	7.0	1	30	12.0/16.0 = 0.75
838	60	M	Neg.		Severe	Present	Torantil	5.0	9	40	4.0/ 6.8 = 0.6
842	51	F	Pos.	H.F.	Moderate	Present	Nil		2	20	16.0/20.0 = 0.80
843	36	F	Neg.		Mild		Nil	3.5	7	16	7.2/12.8 = 0.60
844	36	F	Pos.		Moderate		Bact. vaccine	8.0	2	10	2.0/ 2.0 = 1.0
856	63	M	Neg.	H.F.	Moderate	Present	Bee venom	6.0	2	30	2.0/ 8.0 = 0.25
857	64	F	Neg.		Severe	Present	Nil	4.0	8	24	13.6/13.2 = 1.0
859	58	F	Pos.		Severe	Present	Bact. vaccine		27	20	14.0/16.0 = 0.90
863	47	F	Pos.	H.F.	Moderate		Bact. vaccine	8.0	6	22	12.0/12.0 = 1.0
910	40	F	Pos.	H.F.	Mild		Bact. vaccine	8.5	7	22	10.8/16.0 = 0.53
B											
839	19	F	Pos.		Moderate	Present	Torantil	2.5	2	16	22.0/ 2.0 = 11.0
840	42	F	Pos.	H.F.	Mild		Bact. vaccine	3.0	5	32	8.8/ 4.0 = 4.7
841	18	M	Neg.	H.F.	Mild		Pollen vaccine	2.0	3	17	10.8/ 4.0 = 2.7
858	56	F	Pos.	H.F.	Moderate		Pollen vaccine	7.5	7	24	20.8/ 4.0 = 5.2
860	51	F	Pos.		Moderate		Nil	8.0	5	36	18.0/12.8 = 1.4
862	52	F	Pos.	H.F.	Moderate	Present	Bee venom	14.0	10	10	22.0/ 4.0 = 5.5
905	29	M	Pos.	H.F., R.	Moderate		Nil	16.0	12	14	6.0/ 4.4 = 1.4

Ecz. = Eczema.

clinical classification so commonly employed, viz., pure allergic, infective, and mixed types. The features of these types from the point of view of the differential sedimentation reaction, are summarized below:

Type I. Pure Allergic Type (Chart 2)
Differential ratio generally above 3.
Percentage deviation commonly negative.
Dotted field enlarged, striped field reduced.

Type II. Bronchial Infective Type (Chart 3)
Differential ratio usually below 1.
Percentage deviation positive and often high, e.g., 20 per cent.
Dotted field normal, striped field increased.

Type III. Mixed Type
Differential ratio commonly between 1 and 2.
Percentage deviation variable, e.g., 30 per cent to −10 per cent.
Dotted and striped fields enlarged.

For the red blood cell sedimentation determinations described in this paper the micromethod employed in the Midhurst Sanatorium (Vide Midhurst Report, 1933-34) was used. It lends itself to routine clinical estimations, requiring only a small volume of blood, and is simple in procedure. The sedimentation tubes are of thick glass, 15 cm. long, 1.13 mm. bore, and are graduated in millimeters. For each test 0.4 c.c. of the same sample of venous blood as used for the vanadate reaction is drawn into a 1 c.c. syringe containing 0.1 c.c. of sterile 3.8 per cent sodium citrate solution, thoroughly mixed in a capsule, sucked into the sedimentation tube, adjusted so that the column is 100 mm. in length, and set up in the rack at room temperature.

After one hour the clear zone is observed, its depth in millimeters being the figures recorded as the sedimentation rate. From the citrated blood a film was prepared from which the eosinophile count was made after staining with Jenner's stain.

MATERIAL AND RESULTS

The series of cases presented in this paper are made up of a group of 50 patients suffering from bronchial asthma, a group of 25 patients suffering from various other nonmalignant conditions uncomplicated by asthma, and a group of 50 patients with definite and moderately advanced malignancy.

The last two groups are taken from the sera examined since the initial communication by one of the present writers (D. L. W.) on the modified vanadate reaction (Jones and Woodhouse) and comprise additional material, since the full charting method was not employed in the previous investigation.

The results of all the analyses were charted according to the method outlined above, and the deviations and ratios so calculated are listed in Tables I to V. Differential sedimentation curves of some typical cases are also included in Charts 4 to 7.

It has been satisfactorily demonstrated by other workers, e.g., Cuttler (1932), Kessler (1936), and Reichel (1936), that there is no specific correlation between neoplasia and the rate of blood corpuscle sedimentation, though there is an increased rate of fall in many cases of malignancy probably due to tissue destruction. Therefore, no sedimentation figures are given for these cases included in Tables IV and V.

DISCUSSION

Of the 50 asthmatics examined in the present investigation 19 had ratios of the type formerly called "malignant," although the probability of undetected cancer among this group was relatively small considering the ages of the patients, etc.

The series of malignant cases in this investigation yielded a very high proportion of ratios "positive for cancer," viz., 86 per cent (Table IV). Possibly this is due in part to the fact that the patients in question were all in a moder-

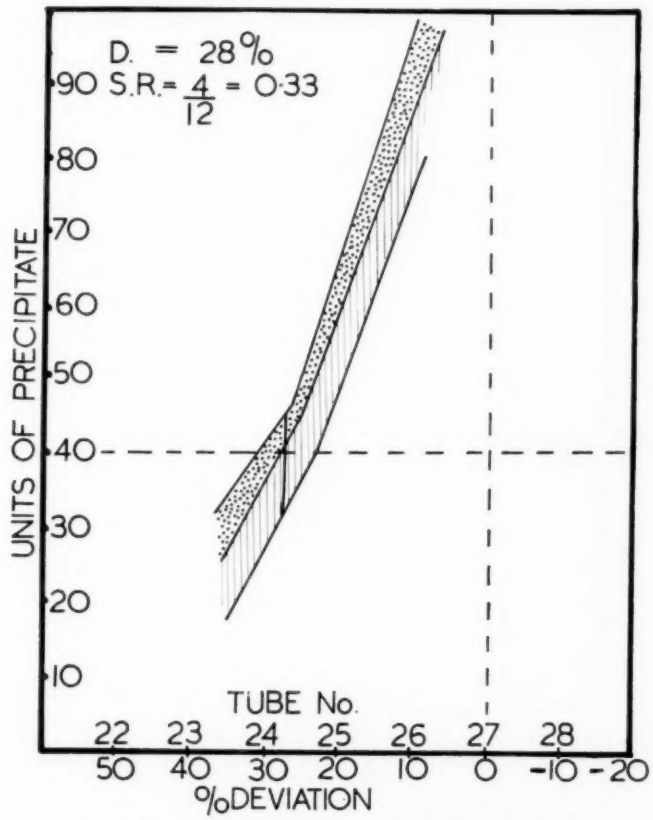

Chart 5.—Nonmalignant case (No. 624, Table IV).

ately advanced, though not terminal, state. The series of nonmalignant cases also has yielded a fairly satisfactory return in that 76 per cent of "negative" returns are recorded (Table V).

In reviewing the clinical data of the asthmatic patients the following points were noted: age and sex, family history of allergy, presence of any other allergic disease, any acute pathologic process in action, and any treatment in course. In 20 cases the tests were carried out prior to any specific treatment.

Tables I, II, and III contain results and data relevant to the asthmatic patients. They have been grouped into three divisions from a consideration of

TABLE III

CLINICAL FEATURES AND LABORATORY FINDINGS

LAB. NO.	AGE	SEX	FAMILY HISTORY	OTHER ALLERGIC DISEASE	SEVERITY OF ASTHMA	CHRONIC BRONCHITIS	TREATMENT	EOSINOPHILE COUNT PER CENT	ERYTHROCYTE SEDIMENTATION RATE IN MM.	PERCENTAGE DEVIATION	SEDIMENTATION RATIO
A											
866	29	F	Neg.		Severe	Chronic	Bact. vaccine	9.0	3	−7	10.0/12.0 = 0.83
886	36	F	Neg.		Moderate		Nil	2.0	2	−10	12.0/10.4 = 1.2
890	21	F	Neg.		Mild		Bact. vaccine	8.5	5	−15	5.2/13.2 = 0.4
895	53	M	Neg.		Mild	Present	Peptone	3.0	4	−17	6.4/ 9.2 = 0.7
897	40	F	Neg.		Moderate	Present	Nil	11.0	4	−7	6.0/10.0 = 0.6
900	41	F	Neg.	H.F., R.	Severe		Nil	2.0	4	−14	6.0/16.0 = 0.4
904	39	F	Pos.	H.F.	Moderate		Pollen vaccine	6.0	2	−14	6.8/ 9.2 = 0.7
915	33	F	Neg.		Severe		Bact. vaccine	6.0	3	−6	16.8/18.8 = 0.9
B											
868	23	F	Neg.		Moderate		Bact. vaccine	5.0	2	−18	27.0/ 1.2 = 22.7
874	9	M	Neg.		Moderate		Nil	5.0	2	−15	18.0/ 6.0 = 3.0
875	22	M	Neg.	R.	Moderate	Present	Nil	5.0	12	−15	12.8/ 8.0 = 1.6
885	33	M	Neg.	H.F.	Moderate	Present	Nil		1	−18	27.2/ 6.0 = 4.5
891	32	M	Pos.	H.F.	Moderate		Nil	9.0	5	−7	22.0/ 8.8 = 2.5
896	34	F	Pos.	H.F.	Mild		Pollen vaccine	8.0	2	−25	15.0/ 5.0 = 3.0
908	20	F		H.F.	Mild		Peptone	11.0	6	−13	16.0/ 4.2 = 3.3

TABLE IV

NONMALIGNANT CASES (PATHOLOGIC)

LAB. NO.	PATHOLOGIC CONDITION	SEDIMENTATION DEVIATION PER CENT	DIFFERENTIAL SEDIMENTATION RATIO
504	Empyema	90	14/29 = 0.5
509	Gastric ulcer	50	4/28 = 0.14
537	Menopausal hemorrhage	24	4.8/ 8 = 0.6
554	? tuberculosis	75	4/16 = 0.25
590	Venereal disease (? cured)	20	6/ 8 = 0.75
601	Ulcer palate (histologically nonmalignant)	45	4/28 = 3.3
602	For repair uterus	80	4.8/20 = 0.28
611	Tuberculosis palate (history nonmalignant)	50	10/20 = 0.5
613	Lead poisoning	38	25/36 = 0.7
623	Rheumatoid arthritis	55	2/28 = 0.07
624	Gastric ulcer	28	4/12 = 0.33
628	Jaundice (relieved)	50	12/18 = 0.7
629	Gastric ulcer	32	20/20 = 1.0
630	Colitis	16	28/30 = 0.9
641	Dysmenorrhea	25	4/36 = 0.11
644	Diabetes	50	12/26 = 0.46
646	Ovarian cyst	30	6/12 = 0.5
650	Gastric ulcer	32	12/14 = 0.8
651	Diabetes	15	20/20 = 1.0
500	Duodenal ulcer	25	48/24 = 2.0
501	Dysmenorrhea	4	46/20 = 23.0
518	Duodenal ulcer	26	56/28 = 2.0
507	Cholecystitis	20	72/14 = 5.2
513	Bronchopneumonia	80	14/14 = 3.5
634	Dysmenorrhea	18	32/28 = 1.1
609	Normal healthy	0	2.4/22 = 0.18
610	Normal healthy	12	20/24 = 0.8

the type of chart obtained. Table I contains those cases in which only slight deviations were evident, the greatest being 8 per cent and the lowest –4 per cent. In Table II the deviations are in every case 10 per cent or greater and all positive. In Table III the sera giving marked negative deviations are set out. In each case these groups have been subdivided into two sections, A and B, the first comprising those in which the sedimentation ratios were approximately equal to or less than unity, and the second containing those with ratios definitely greater than unity.

This method of presentation of results should, if the claims of Coke can be accepted as generally valid, distribute the cases into the clinical groups: viz., allergic, infectious, and mixed. Employing this method of interpretation, it is obvious that the cases included in Table IIA and those in Table IIIB should in the main coincide respectively with the "bronchial infective" and "pure allergic" types of asthma. However, of the 12 cases in Table IIA, only 5 could, on clinical grounds, be found to possess any similarity to the "bronchial infective" type of asthma, and of the 7 cases in Table IIIB, only 3 showed any degree of clinical correspondence. Clinically there seemed to be no marked difference between those cases in the two groups just mentioned which failed to coincide and those in other groups, such as Tables IA and IB.

TABLE V

MALIGNANT CASES

LAB. NO.	PATHOLOGIC CONDITION	SEDIMENTATION DEVIATION PER CENT	DIFFERENTIAL SEDIMENTATION RATIO
502	Carcinoma cervix	25	26/15 = 1.7
503	Carcinoma breast	18	44/12 = 3.7
505	Epithelioma penis	10	88/12 = 7.3
522	Carcinoma breast	30	30/12 = 1.9
526	Carcinoma thyroid	10	38/10 = 3.8
528	Carcinoma breast	25	46/16 = 2.9
530	Carcinoma stomach	90	15/12 = 1.25
540	Carcinoma bronchus with secondaries	90	24/16 = 1.5
546	Adenocarcinoma pelvis	100	12/10 = 1.2
548	Sarcoma femur	10	42/20 = 2.1
558	Carcinoma stomach	10	40/24 = 1.7
562	Carcinoma breast	20	46/14 = 3.3
566	Carcinoma stomach	90	32/19 = 2.2
578	Osteogenic sarcoma	20	44/24 = 1.8
587	Spheroidal cell carcinoma breast	25	46/26 = 1.8
592	Carcinoma cervix	32	28/12 = 2.3
615	Carcinoma stomach	60	40/ 4 = 10.0
616	Carcinoma colon	80	24/12 = 2.0
622	Carcinoma stomach	50	40/ 4 = 10.0
627	Carcinoma lung	60	24/ 4 = 6.0
618	Carcinoma stomach	40	23/ 2 = 11.5
550	Recurrent carcinoma breast (glands)	30	4/16 = 0.25
551	Carcinoma cervix	20	10/30 = 0.33
647	Epithelioma vulva	36	4/24 = 0.17
626	Carcinoma cervix	40	33/40 = 0.8
515	Carcinoma breast	14	40/32 = 1.25
519	Carcinoma perineum	50	28/ 5.0 = 5.6
520	Carcinoma colon	70	16/12 = 1.3
521	Carcinoma breast	10	26/14 = 1.9
525	Epithelioma tongue	22	24/ 4 = 6.0
527	Epithelioma face	10	46/27 = 1.7
531	Epithelioma tongue	34	36/18 = 2.0
532	Epithelioma tongue (recurrent)	30	52/30 = 1.7
533	Carcinoma breast	10	76/11 = 4.8
534	Epithelioma floor of mouth	24	46/20 = 2.3
535	Carcinoma lung	55	30/ 2 = 15.0
542	Epithelioma tongue	18	60/12 = 5.0
543	Carcinoma breast	18	26/12 = 2.2
545	Carcinoma ovary	52	16/14 = 1.1
553	Epithelioma ear	40	36/28 = 7.3
563	Carcinoma breast	40	40/12 = 3.3
567	Epithelioma tongue	55	33/ 6.8 = 4.9
555	Epithelioma nipple	16	28/16 = 1.8
568	Epithelioma face	33	41/24 = 1.7
572	Carcinoma breast	30	65/ 6 = 10.9
604	Epithelioma face	25	46/46 = 1.0
625	Carcinoma cervix	50	32/12 = 2.7
559	Epithelioma tongue	10	28/40 = 0.7
561	Epithelioma lip	10	36/44 = 0.8
633	Carcinoma cervix	35	16/24 = 0.7

On the contrary an uncomplicated case of hay asthma (880) examined when symptoms were present did not show the typical serologic findings which according to Coke characterize the "pure allergic" group. In another instance a patient (883), who on clinical grounds would be placed in the "bronchial infective" group, gave a normal differential sedimentation reaction.

But although we were not able to distinguish by means of the sedimentation data between the clinical types as definitely as would be expected from the description by Coke, yet the cases studied presented distinguishing features as a whole. Thus among the 75 pathologic cases not one instance of a nega-

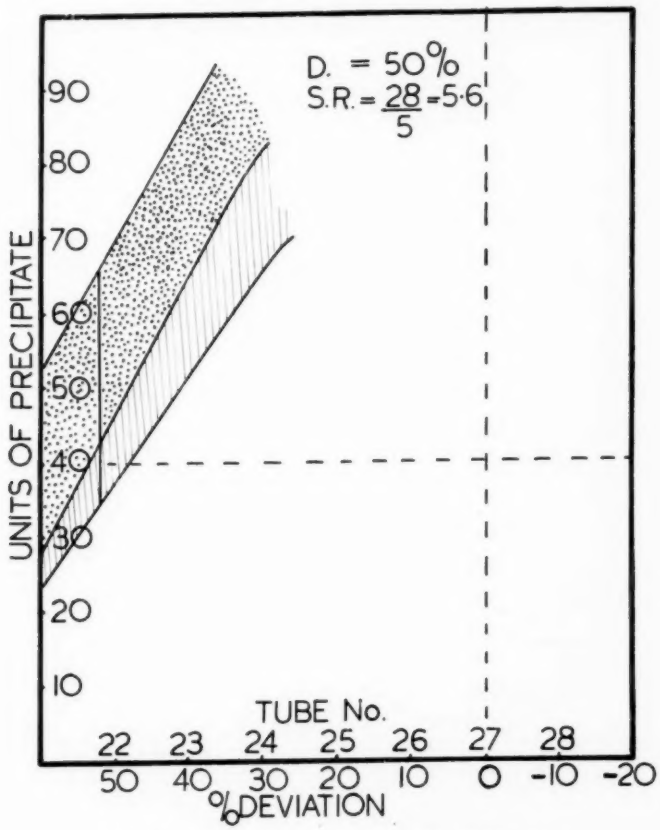

Chart 6.—Malignant case (No. 519, Table V).

tive serum sedimentation deviation was encountered while among the asthmatics no less than 15 occurred with definite negative deviations, and 11 of these to the extent of 10 per cent or more.

It did not seem possible, however, to correlate the individual results of the differential sedimentation tests with the clinical picture in a manner which was thoroughly convincing.

Also examination of Tables I, II, and III shows that it is difficult to correlate the blood sedimentation rate with the percentage deviation. Thus definite acute infections (864, 876, Table I) with fast sedimentation rates had normal deviations while normal sedimentation rates (Table II) were accompanied by

high deviations. Again in Table III, where high negative deviations are grouped, there is no evidence of very slow blood sedimentations.

Since negative deviations seem to be so characteristic of asthma, it was considered possible that a relationship might exist between these and the eosinophile count. The eosinophile counts in Tables I, II, and III were not in all cases made from the same samples of blood which were employed for the blood sedimentation rate and differential sedimentation reaction estimations. Usually, however, they were prepared within a few weeks of these tests.

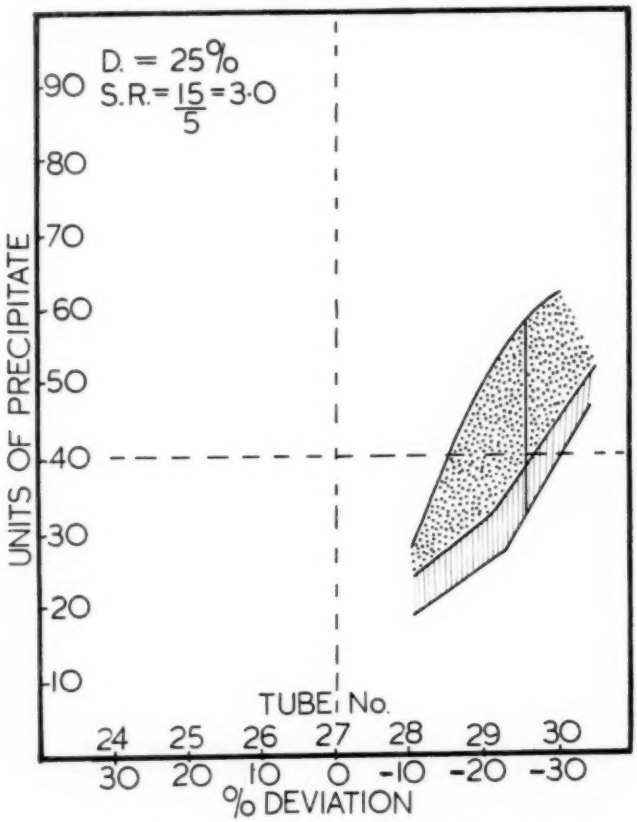

Chart 7.—Asthmatic case (No. 896, Table IIIB).

The average eosinophile count for the cases with negative deviations (Table III) is 6.5, and for those cases with positive deviations (Table II) is 6.7. In the present circumstances so small a difference appears of no significance. It is thus not possible to correlate the type of deviation with the eosinophile count.

SUMMARY AND CONCLUSION

The differential serum sedimentation figures have been determined for a group of 50 asthmatic patients, a group of 50 malignant patients, and 25 patients suffering from nonmalignant diseases, and the corresponding charts drawn for the values. In the asthmatic series determinations of the red blood corpuscle sedimentation rates and the eosinophile counts were also made.

The differential serum sedimentation tests did not appear to give definite information from the point of view of clinical classification of the asthmatic patients. About 30 per cent of these, however, showed a very definite negative deviation, a feature not observed in other groups.

It was not found possible to correlate the degree of eosinophilia with the sedimentation deviation or with the blood corpuscle sedimentation rate.

The vanadate sedimentation estimations have been carried out as a part of the inquiry into serological reactions, especially in relation to malignant disease by one of the authors (D.L.W.) under the auspices of the British Empire Cancer Campaign, Birmingham Section. For facilities in connection with the Asthma Clinic Patients, our best thanks are due to Professor W. H. Wynn.

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TRICHOSTRONGYLUS COLUBRIFORMIS IN THE HUMAN APPENDIX*

Report of a Case in Louisiana

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A NEMATODE was discovered in the appendicial content of an 11-year-old white female, native of Louisiana, whose appendix had been removed because of recurrent attacks of abdominal pain. Professor E. C. Faust, of the Tulane University School of Medicine, identified the parasite as an adult male *Trichostrongylus colubriformis*. Although members of the Trichostrongyloidea are commonly present in the intestinal tract of sheep and goats in North America, no instance of human infection with this parasite has been previously reported in the Western hemisphere. Human parasitism by *T. colubriformis* has been observed in Egypt, India, Armenia, and Australia, particularly among individuals in sheep and goat raising localities. Although we do not believe that the parasite was responsible for the complaints of the patient, we feel that its discovery in the intestinal canal of a human being should be recorded.

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REPORT OF CASE

On August 15, 1937, P. S., an 11-year-old white female, experienced a sudden cramping abdominal pain which localized to the right of the umbilicus. She soon became nauseated and vomited several times. The administration of a mild laxative caused no alteration of the symptoms. The pain persisted as a dull discomfort with knifelike exacerbations, but never radiated or became localized in the right lower quadrant. She was admitted to the Charity Hospital on August 29. She had experienced headache, vertigo, and mild attacks of abdominal pain for about one year, which was thought to be caused by a chronic recurrent appendicitis. On admission the physical examination revealed a well-nourished and well-developed ambulatory child, with a normal temperature, pulse rate, respiratory rate, and blood pressure. Except for slight tenderness and rigidity of the abdomen, about 5 cm. to the right of the umbilicus, the physical findings were normal. An appendectomy was performed on August 31. The surgeon saw no evidence of an acute inflammation or periappendicial adhesions. The

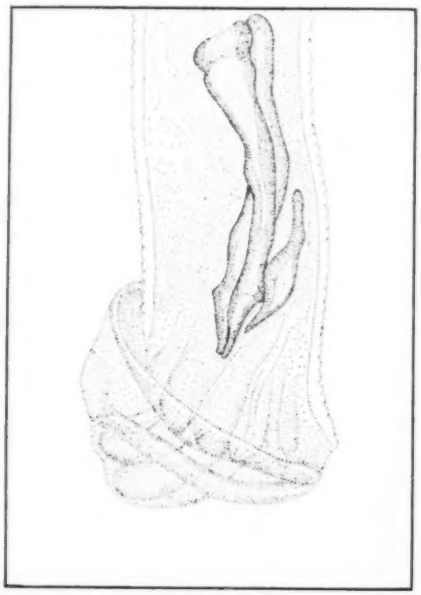

Fig. 1.—Drawing of the bursa of *T. colubriformis*. Note the characteristic copulatory spicules.

postoperative course was uneventful, and she had no complaints at the time of her discharge on September 16. On December 8 she denied any recurrence of the abdominal pain, but had had a few attacks of vertigo.

Laboratory Study.—The examination of the appendicial content revealed two adult male *Enterobius vermicularis* and single adult male *Trichostrongylus colubriformis*. Examination of the stool obtained after a saline enema revealed one rhabditiform larva of *Strongyloides stercoralis*. Another stool examination made following carbon tetrachloride administration was negative. No Trichostrongylus ova were found. The blood picture on September 8, 1937, was normal, except for a 7 per cent eosinophilic leucocyte count. A stool obtained on December 8 revealed no ova or parasites.

Pathologic Report.—Macroscopically the appendix appeared normal. Microscopically small patches of plasma cell and to a less extent macrophagic infiltration directly beneath the mucosa were observed. This response was not present in any other part of the appendix. There was no evidence of an acute inflammation. Several cross sections of *Enterobius vermicularis* were observed in the lumen.

Since several of the standard textbooks of parasitology contain excellent descriptions of the Trichostrongyloidea, we are only submitting a drawing of the bursa of the specimen, showing the characteristic copulatory spicules.

DISCUSSION

According to Craig and Faust (1937) several hundred worms are necessary to provoke marked clinical manifestations. Our case showed no anemia or emaciation and undoubtedly was a light infection, possibly only a single worm being present. In view of the fact that two other parasitic species were discovered in the intestinal content, we were unable to hold the Trichostrongylus responsible for the mild chronic inflammation in the appendix. The patient's symptoms and signs suggested an appendicial colic and, although the parasite may have been a factor in the production of spasm, the Enterobius alone could initiate these symptoms. No definite information could be obtained regarding the source of the infection. The patient lives in the city and her only contacts with ruminants were those at the municipal park and an occasional visit in the country where very few sheep or goats are raised.

SUMMARY

1. An instance of *Trichostrongylus colubriformis* in the human appendix is reported.

2. No etiologic relationship between the parasite and the appendicial inflammation can be established.

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MASSIVE DOSES OF VITAMIN D IN CHRONIC ARTHRITIS: ITS EFFECT ON CALCIUM METABOLISM*

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THE first paper on the use of vitamin D in massive doses appeared in September, 1935.[1] In that publication Dreyer and Reed reported the results of two years' experience in the use of massive doses of vitamin D in 10 cases of infectious arthritis, 24 cases of atrophic arthritis, 14 cases of hypertrophic arthritis, 4 cases of menopausal arthritis, 3 cases of mixed arthritis, 5 cases of miscellaneous joint cases, and 7 cases of so-called arthralgia. Of this entire group 44 patients manifested clinical improvement, 13 patients were clinically unimproved, and in 10 patients the results were uncertain. These clinical investigators employed 200,000 to 1,000,000 units daily of vitamin D. The usual dose was 200,000 to 300,000 units daily.

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The next report, inspired by the first publication noted above, appeared in April, 1936.[2] Vrtiak and Lang reported the result of therapy with massive doses of vitamin D in 20 cases of atrophic arthritis in their study. They employed 150,000 to 300,000 units of vitamin D daily. The duration of treatment varied from three to ten months. Twelve of the patients showed a varied degree of clinical improvement. Eight of the 20 cases showed no improvement. The third report concerning this method of therapy appeared in October, 1936.[3] Wyatt and his co-workers reported on the use of 200,000 to 300,000 daily units of vitamin D in the treatment of 40 patients having atrophic arthritis. Eight of this group showed definite clinical improvement. The treatment failed in 24 cases. It was necessary to abandon the treatment in 8 cases because of unfavorable reactions. Thus clinical improvement has been reported in 46.7 per cent of 137 cases of arthritis, of which the majority was the atrophic type. More recently, Livingston[4] has reported good results by combining fever therapy with massive doses of vitamin D.

None of these investigators has reported any detailed study of the effects of massive doses of vitamin D on the serum calcium or serum phosphorus. They have been concerned chiefly with the clinical results. The effect of such therapy on the serum calcium and phosphorus was undertaken in this investigation.

Though much remains to be learned about normal calcium and phosphorus and even more is unknown of abnormal calcium-phosphorus metabolism, from the maze of calcium-phosphorus studies, several known facts have become established. In a normal subject the blood calcium is stabilized at 10 mg. per 100 c.c., the variation being 9 to 11.5. The serum phosphorus is stabilized in the neighborhood of 3 to 4 mg. per 100 c.c. The various factors which maintain these normal values and which upset them in a diseased state are not entirely known. What was thought to be one substance as regards a normal serum calcium is now thought to be three or four physiochemical divisions of this element. The previously accepted concept of these three states was that the serum calcium was made up of (1) a nondiffusible, nonionized fraction which was in loose combination with a serum protein (about 5 mg. per 100 c.c.); (2) a diffusible, nonionized fraction (about 3 mg. per 100 c.c.); and (3) a diffusible ionized fraction (about 2 mg. per 100 c.c.). The more recent concept divides the serum calcium into four fractions: two diffusible and two nondiffusible portions. McLean and Hastings[5] are of the opinion that two of these fractions, the free ionized calcium and the calcium bound to the serum protein, are important to human economy.

Four or five factors operate to affect the serum calcium and phosphorus in a normal subject; these are (1) the calcium intake, (2) the phosphorus intake, (3) the hormone from the parathyroid gland, (4) vitamin D, and (5) the ferment phosphatase. There are some writers[6] who believe that the ingestion of calcium per se has no effect on the blood calcium; others believe contrawise. The latter group[7] claim that the period in which the blood is drawn for analysis after the calcium ingestion is most important. The phosphorus intake has a pronounced effect on the blood calcium. The ingestion of phosphorus in large doses in the form of sodium acid phosphate, has the following sequelae: (1) secretion of the excess phosphate in the urine, (2) a fall in an elevated serum

calcium, (3) an increase in output of fecal calcium, and (4) a fall in the calcium output in the urine. These sequelae result from the formation of insoluble calcium salts in the intestine.

The parathyroid hormone[8] raises the serum calcium and lowers the serum phosphorus; it increases the excretion of calcium and phosphorus in the urine; it increases the ionized calcium in the blood. The increased demand for calcium is either withdrawn from bony tissue or else supplied from a large amount of ingested calcium. Cantarow[9] has shown that vitamin D in excessive amounts causes a rise in the level of blood calcium or phosphorus or both. The extra calcium may come from increased absorption from the intestine, from better calcium utilization, or by withdrawal from the osseous tissue. The exact manner in which vitamin D brings about these changes is unknown. Some[10] believe that vitamin D stimulates the parathyroid tissue directly. However, there are many differences in the pharmacodynamics of vitamin D and parathyroid hormone which make the above hypothesis unlikely. The reader should refer to more detailed studies of calcium metabolism on this controversial subject.

Vitamin D also affects the ferment phosphatase, which is an ubiquitous substance in human economy. It has been found in such diverse tissues as the intestinal mucosa, kidney, bone, spleen, liver, pancreas, lung, cerebrum, muscle, milk, urine, bile, and others. It is chemically capable of splitting or hydrolyzing both aliphatic and aromatic esters of monophosphoric acid. Vitamin D has the power of restoring this ferment to the bone matrix where it eventually brings about precipitation of tricalcium phosphate for bone formation.

Our study concerns itself with the clinical effects observed in the use of massive doses of vitamin D in the treatment of chronic arthritis and the possible relationship of this effect to the changes in the blood calcium. We have administered this substance in usual doses of 160,000 U.S.P. units daily to 40 cases of chronic arthritis. Of the 29 cases of atrophic arthritis treated, 10 showed clinical improvement and 19 showed no improvement whatsoever. Of the 7 cases of hypertrophic arthritis treated, 2 showed clinical improvement, the results were questionable in one case, and failed in 4 cases. One case of Still's disease treated showed very definite improvement. Two cases of joint disease of unknown type, one of which showed marked osteoporosis around the shoulder girdle, and one case of arthralgia were definitely improved. One case of Marie-Strümpell arthritis showed no clinical improvement by this method of treatment.

Thus, clinical improvement was noted in 14 cases or 35 per cent of the total treated; 26 cases or 65 per cent were unimproved. Three cases of the latter group were definitely aggravated. Of the 29 cases of atrophic arthritis improvement was noted in 10 cases or 34 per cent.

Initial serum calcium and phosphorus determinations were made in 32 cases. The average serum calcium in 25 cases of atrophic arthritis was 11.50 mg. per 100 c.c. Seventeen of this group had calcium values above 11 mg. Eight of this group had calcium values of 12 mg. and more. The phosphorus determination in 23 cases of atrophic arthritis was normal with the exception of two instances, in which the readings were 2.7 and 2.9 mg., respectively. The average age of the male atrophic arthritic studied was thirty-nine years, the range being

TABLE I

TABULAR STUDY OF DETAILED CALCIUM AND PHOSPHORUS DETERMINATIONS IN TWELVE CASES

CASE	HEMATOLOGY	CULTURES	INITIAL CALCIUM AND PHOSPHORUS	EFFECT MASSIVE DOSES VITAMIN D
62-year-old white female with marked osteoporosis of shoulder girdle. Pain in shoulder 18 months.	R.B.C. 3,980,000 Hb. 76 per cent Sahli W.B.C. 6,000 Sed. rate 0.62 Stabnuclears 6 per cent	Throat 60 per cent *Strep. hemolyticus*	Ca 11.7	At the end of 3 weeks treatment with daily doses of 160,000 units vit. D, Ca 13.5, P 4.9. No improvement. After 6 weeks such medication no improvement
43-year-old white male with abduction of arms limited to 20 degrees. Painful wrists and shoulders for 18 months.	R.B.C. 4,860,000 Hb. 94 per cent Sahli Sed. rate 1.55 Stabnuclears 3 per cent	Throat negative for *Strep. hemolyticus*. Skin tests positive for *Strep. viridans*	Ca 10.6 P 4.7	At the end of 2 weeks treatment with daily doses of 160,000 units vit. D, Ca 13.1, P 4.3. Patient able to use both shoulders in all ranges of action
33-year-old white female with typical atrophic arthritis of major joints of body for 8 months. No clinical results with vaccine therapy	Sed. rate 0.52 Stabnuclears 8 per cent R.B.C. 4,270,000 Hb. 86 per cent Sahli W.B.C. 8,000	Dental abscess *Strep. viridans*	Ca 10.7 P 4.2	No improvement at the end of 3 weeks treatment with daily doses of 160,000 units vit. D; 2 months later Ca 13.1. Definite clinical improvement
65-year-old white male with multiple joint pains for 6 years. Undiagnosed type of arthritis	R.B.C. 4,750,000 Hb. 94 per cent Sahli W.B.C. 8,000 Sed. rate 0.34 Stabnuclears 6 per cent	Throat negative for *Strep. hemolyticus*	Ca 9.7 P 4.8	At the end of 3 weeks treatment with daily doses of 160,000 units vit. D, Ca 10.8, P 2.8. Clinical improvement marked. 4 months later Ca 12, P 3.4. Improvement maintained
33-year-old white female with typical atrophic arthritis of several years' duration	Sed. rate 0.40 Stabnuclears 9 per cent R.B.C. 3,360,000 Hb. 67 per cent Sahli	Throat negative for *Strep. hemolyticus*	Ca 10.3 P 4.7	At the end of 3 weeks treatment with daily doses of 160,000 units vit. D, Ca 13.3, P 4.1; 1 month later Ca 13, P 3.9. Clinical improvement marked. 1 month later Ca 9.3, P 3.6; 1 month later Ca 11.8, P 3.2. Clinical improvement marked. 2 months later developed acute exacerbation and for the first time had flexion deformity of right elbow

32 to 49 years. The average age of the female atrophic arthritic was 45.3 years, the range being 32 to 79 years. The average blood calcium in 7 cases of hypertrophic arthritis was 11.2 mg. per 100 c.c. Two had values of 12 mg. or more and 5 of the group had a value about 11 mg. The average blood phosphorus in 6 cases of hypertrophic arthritis was 4.20 mg.

The finding of high normals of serum calcium and even hypercalcium values in chronic arthritis, particularly in the atrophic type, in this series of cases

TABLE I—CONT'D

CASE	HEMATOLOGY	CULTURES	INITIAL CALCIUM AND PHOSPHORUS	EFFECT MASSIVE DOSES VITAMIN D
73-year-old white female with flexion deformity of fingers and marked osteoporosis of hands	R.B.C. 4,420,000 Hb. 88 per cent Sahli Uric acid 2.7 Sed. rate 1.05 Stabnuclears 14 per cent	Negative throat for *Strep. hemolyticus*	Ca 13.2 P 3.8	At the end of 1 week treatment with daily doses of 160,000 units vit. D, Ca 11.2, P 3.7. Improvement marked. At the end of 1 month Ca 13.6, P 3.5. At the end of 2 months Ca 13.2, P 4.2. 1 month later Ca 12.4, P 4.4. Clinical improvement maintained. Another 3 months and patient had regained complete motion of hands. Vit. D stopped. 3 months later Ca 11.5, P 3.9. Patient feeling fine. Another 4 months Ca 12.4, P 3.9. Vit. D restarted. 3 months later Ca 11.7, P 4. Clinical improvement maintained. No change in bone density
35-year-old white female with typical atrophic arthritis of several years' duration	R.B.C. 3,960,000 Hb. 79 per cent Sahli Sed. rate 0.98 Stabnuclears 9 per cent	Tonsils negative for *Strep. hemolyticus*	Not done	At the end of 3 weeks treatment with daily doses of 160,000 units vit. D, Ca 12.2, P 3.3. 2 months later Ca 12, P 4.1. No clinical improvement. Another 2 months of treatment and joints definitely worse
32-year-old white female with typical atrophic arthritis for years	R.B.C. 4,000,000 Hb. 70 per cent Sahli Sed. rate 0.80	Throat 60 per cent *Strep. viridans*	Ca 10.9 P 3.9	At the end of 1 month treatment with daily doses of 160,000 units vit. D, Ca 12.3, P 4.1. Joint condition definitely worse
59-year-old white female with advanced hypertrophic arthritis of spine	R.B.C. 4,420,000 Hb. 87 per cent Sahli W.B.C. 16,200 Sed. rate 0.49 Stabnuclears 10 per cent	Throat 75 per cent *Strep. viridans*	Ca 8 P 3.6	At the end of 3 weeks treatment with daily doses of 160,000 units vit. D, Ca 10.4, P 4. No improvement. At the end of another 3 months treatment Ca 12, P 4.1. No improvement

needs further explanation. It is true that no agreement exists as to whether or not a normal or abnormal blood calcium is characteristic of chronic arthritis. Some investigators[13] have reported frequent elevations of the blood calcium in various forms of chronic arthritis; others[14] have found normal calcium values in 97 per cent of chronic arthritics studied. The explanation of these differences in opinion probably can be explained on the vicissitudes of the pathologic process

TABLE I—CONT'D

CASE	HEMATOLOGY	CULTURES	INITIAL CALCIUM AND PHOSPHORUS	EFFECT MASSIVE DOSES VITAMIN D
44-year-old white female with typical atrophic arthritis	R.B.C. 4,310,000 Hb. 82 per cent Sahli W.B.C. 7,100 Sed. rate 0.70 Stabnuclears 5 per cent	Throat negative for *Strep. hemolyticus*	Ca 12	At the end of 3 weeks treatment with daily doses of 160,000 units vit. D, Ca 12.1, P 4.1. No improvement. At the end of another month Ca 15.3, P 3.5. At the end of 2 weeks marked improvement. Another 2 weeks Ca 12.2, P 3.5. Joints quite achy. (No Vit. D for 2 weeks previous.) Vit. D restarted and at the end of another 2 months Ca 13.1, P 3.7. Marked clinical improvement
38-year-old white female with moderately advanced hypertrophic arthritis	R.B.C. 4,310,000 Hb. 84 per cent Sahli W.B.C. 8,300 Sed. rate 0.24 Stabnuclears 3 per cent	Throat 100 per cent *Strep. hemolyticus*	Ca 13	At the end of 3 weeks treatment with daily doses of 160,000 units vit. D, Ca. 12.1, P 4.5. No joint pains. Another 3 weeks Ca 12, P 3.3. Feeling fine. 1 month later Ca 11.3, P 4. Clinical improvement maintained
63-year-old white male with infectious arthritis 4 months' duration	R.B.C. 4,960,000 Hb. 95 per cent Sahli W.B.C. 7,200 Sed. rate 0.38 Stabnuclears 5 per cent	Dental abscess *Strep. viridans*	Ca 11.2 P 3.9	At the end of 2 weeks treatment with daily doses of 160,000 units vit. D, Ca 11.6, P 4.5. No improvement. 6 weeks later Ca 12.2, P 4.1. Very slight improvement

characteristic of chronic arthritis, particularly the atrophic type. The writer has found marked variations in calcium values in untreated atrophic arthritic patients. These variations may occur with changes in the pathologic processes which cause characteristic decalcification in the articular and juxta-articular position.

An interesting physiopathologic process peculiar to atrophic arthritis is that the decalcification is usually limited to the epiphyseal end of the bones. Seldom does one observe such changes in the diaphysis. The decalcification or osteoporosis is sufficient at times to soften the bone so that the body weight induces what may appear to be a compression fracture. The localized demineralization is evidently due to the localized highly vascularized granulation tissue. This belief is suggested by Jones and Roberts.[11]

Detailed calcium and phosphorus determinations were carried out in twelve of the treated cases, varying in periods of three weeks to one and one-half years. This study was performed to determine whether or not there was any possible

specificity of action which might be ascribed to this method of therapy. Sixty-eight calcium determinations and 64 phosphorus studies were made on this group. The diets in all these cases were similar. All received 1000 c.c. of milk daily, 4 cups of 5 per cent and 10 per cent vegetables, 240 gm. of 10 per cent fruit, 60 gm. of whole wheat bread, 1 gm. protein per kg., and sufficient butter fat to make up the caloric requirement.

A study of Table I suggests that massive doses of vitamin D have the following sequelae on the blood calcium. It will lower a high serum calcium; it will raise a low or normal serum calcium to a higher level and then, after continued administration, will again decrease the hypercalcium level. The clinical improvement or nonimprovement or even aggravation of existing joint symptoms had no bearing on the change of blood calcium; for in some cases with the production of a hypercalcemia, clinical improvement would be noted, and in others an opposite effect or no improvement would occur. The effect on the serum phosphorus was less marked.

It appears that the effect of massive doses of vitamin D in chronic arthritis is nonspecific, and that the improvement noted with this medication would fall in line with the many other remedies employed in the nonspecific treatment of chronic arthritis.

Although massive doses of vitamin D were administered in 40 cases of chronic arthritis in periods, ranging from several weeks to one and one-half years, and were also given to a child aged 3 to 4 years in doses of 160,000 units daily, no untoward results occurred. Even though the author feels that no specific virtues exist in such medication, at the same time he feels that its toxicity has been overemphasized. The above statement is not meant to imply that larger doses than employed here may not be dangerous or even fatal. The diets in all our cases were controlled. If the calcium intake had been increased, or acid substances such as ammonium chloride given, hypervitamosis most probably would have resulted. The clinical investigator should beware of the following symptoms in the use of massive doses of vitamin D: nausea, anorexia, vomiting, cramps, diarrhea, thirst, and frequent urination. Also peripheral nerve changes, pain in muscles and joints, headaches, haziness, and vertigo are all danger signals of hypervitamosis D. The drug should be stopped as soon as any of these symptoms occur. It may be restarted ten to fourteen days later.

The concentrated vitamin D used in this study was supplied through the courtesy of the experimental medical department, Parke-Davis & Co.

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STUDIES ON THE ANEMIA OF PREGNANCY IN GASTRECTOMIZED AND NORMAL DOGS*

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THE anemias of pregnancy have been recently reviewed by several authors.[1-7] They reported that "secondary" anemia, a hypochromic microcytic anemia, was the most common of the true anemias of pregnancy. This anemia was found to occur in from 30 to 40 per cent of all pregnant women.

Ivy, Morgan and Farrell[8] in 1931 reported the occurrence of a spontaneous anemia in completely gastrectomized dogs. They also reported a few instances of anemia of pregnancy in gastrectomized dogs. The spontaneous anemia and the anemia of pregnancy were "secondary" anemias. These authors concluded that in the gastrectomized dog the factor of safety in digestion was so impaired that the added strain of pregnancy caused an anemia. To further investigate this anemia of pregnancy in the gastrectomized dog, the following studies were made.

Five gastrectomized dogs were observed through a total of fifteen pregnancies. A pregnancy which occurred after a five-foot enterectomy in one gastrectomized dog and a pregnancy which occurred after a six-foot enterectomy in another gastrectomized dog were studied. As a control series, twelve normal dogs were observed through twelve pregnancies.

METHODS

The five gastrectomized dogs used in this study were under observation in this laboratory for periods varying from two to seven years. When this study was first undertaken, hematologic procedures were confined to determination of red blood count and hemoglobin; but these studies were later extended to include determinations of hematocrit, blood volume, and, in two cases, mean cell diameters. All animals, including the gastrectomized dogs, received the

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same stock laboratory diet made up of boiled hamburger, white bread, prepared cereal, milk, and cod-liver oil.

Hematologic Procedures.—With the exception of the first seven pregnancies observed in the gastrectomized dogs, blood examinations consisting of red blood count, hemoglobin by the Newcomber method, hematocrit by Rosahn's method,[9] and blood volume by the method of Hopper and associates[10] were made at intervals of approximately ten days. Examinations were made more frequently just prior to and just following delivery.

RESULTS

Standard values for red blood count, hemoglobin, and hematocrit established for normal dogs in our laboratory compare favorably with those accumulated by Wintrobe and co-workers.[11] For purposes of conciseness and clarity, arbitrary limits for slight, moderate, marked, and severe anemia were established. The definitions of these limits follow:

ANEMIA	R.B.C.	HB.	HEMATOCRIT	FRACTION OF NORMAL
Normal	7.00	15.00	46.50	6/6
Slight	5.83	12.50	38.70	5/6
Moderate	4.67	10.00	31.00	4/6
Marked	3.50	7.50	23.20	3/6
Severe	2.33	5.00	15.50	2/6

Gastrectomized Dogs.—Gastrectomized Dog No. 11 was observed during eight pregnancies. In five pregnancies (No. 11-1, 2, 3, 6, 7) hypochromic anemia gradually developed in the last thirty days of pregnancy to become marked during the week preceding delivery. In one pregnancy, No. 11-4, a marked normochromic anemia and in another pregnancy, No. 11-5, a severe hypochromic anemia were present at term. The eighth and last pregnancy observed in this dog occurred after a five-foot enterectomy. This pregnancy was terminated by a fatal hemorrhage caused by uterine rupture from pressure necrosis.

In pregnancy No. 11-3 daily feeding of one vial of liver extract No. 343 (10 c.c. from 50 gm. liver) Lilly instituted on the sixteenth ante-partum day resulted in a marked increase in red blood count, but had no effect upon the hemoglobin level of the blood. In pregnancy No. 11-4 daily feeding of 10 gm. of liver extract No. 55 Lilly instituted upon the sixteenth ante-partum day had no demonstrable effect on the blood picture.

In pregnancy No. 11-5 the blood volume at term was increased by approximately 240 c.c. and the total circulating hemoglobin decreased by approximately 50 gm.; in pregnancy No. 11-6 the blood volume was increased by approximately 350 c.c. and the total circulating hemoglobin decreased by approximately 25 gm.; and in pregnancy No. 11-7 the blood volume was increased by approximately 100 c.c. and the total circulating hemoglobin decreased by approximately 25 gm.

Gastrectomized Dog No. 26 was observed during three pregnancies. In pregnancy No. 26-1 a marked hypochromic anemia, in pregnancy No. 26-2 a

CONDENSED PROTOCOLS

Gastrectomized Dog No. 11

NO. OF PREG. AND TIME AFTER OPERATION	DAYS ANTE OR POST PARTUM	WT. LBS.	R.B.C. MILLIONS	HB. GM.	HEMATOCRIT	COLOR INDEX	BLOOD VOLUME	TOTAL HB.	MISCELLANEOUS
1st	55 a.p.		5,70	13.10		1.07			Premature delivery; 6 or 7 wk. pregnant
3 mo. p.o.	0		3,44						
	15 p.p.		3,90	8.15		0.97			
	64 p.p.		5,50	11.80		1.00			
2nd	49 a.p.		6,00	12.80		1.00			Premature delivery; dead fetuses
1 yr. p.o.	20 a.p.		4,90	8.70		0.83			
	0		4,70	7.80		0.78			
	15 p p.		5,50	9.70		0.82			
	79 p.p.		6,70	14.40		1.00			
3rd	46 a.p.		6,80	11.60		0.80			Term; 11 pups
1 yr. 6 mo. p.o.	16 a.p.		4,20	8.00		0.89			
	1 a.p.		6,90	7.78		0.53			1 vial liv. ext. No. 343 daily
	15 p.p.		5,40	9.50		0.82			
	81 p.p.		4,54	8.80		0.90			
4th	45 a.p.		5,70	12.05	40.50	0.99			Term; 10 pups
2 yr. 1 mo. p.o.	16 a.p.		4,10	10.20	26.20	1.16			
	0		4,20	8.70	22.80	0.97			1 vial liv. ext. No. 55 daily
	21 p.p.		4,60	10.10	30.40	1.03			
	42 p.p.	40¾	6,73	13.10	34.60	0.91			
5th	55 a.p.	40½	6,05	12.56	29.90	0.97	1178	148	Premature delivery; dead fetuses
2 yr. 7½ mo. p.o.	10 a.p.	43½	4,49	8.54	27.50	0.89	1314	112	
	1 a.p.	49¾	4,30	6.97	26.00	0.75	1420	99	
	18 p.p	42¼	5,29	11.10	28.90	0.98	1223	136	
	37 p.p.	46	6,91	12.90	41.10	0.87	1255	162	
6th	59 a.p.	46¾	6,98	12.63	34.90	0.84	1194	151	Premature delivery; dead fetuses
3 yr. 1 mo. p.o.	15 a.p.		5,69	10.03		0.82			
	0	48	4,83	8.42	25.90	0.81	1450	122	
	13 p.p.		4,69	8.21		0.82			
	72 p.p.	47¾	6,52	11.38	36.50	0.82	1230	140	
7th	59 a.p.		5,57	12.77		1.07			Term; 4 pups
4 yr. 2½ mo. p.o.	16 a.p.		5,02	11.19		1.10			
	0		4,55	8.90		0.91			
	1 p.p.	47	4,58	8.90	32.30	0.91	1302	116	
	17 p.p.		6,03	9.87		0.77			
	47 p.p.		7,85	11.19		0.67			

Seven years after gastrectomy No. 11 was subjected to a 5 ft. enterectomy.

Composite of 4 determinations before operation		44	5,64	11.21	37.75	0.93		
8th	59 a.p.	35	4,60	11.48	37.00	1.16	1160	133
181 days after enterectomy	26 a.p.	32¾	4,03	9.85	34.00	1.14	1090	107
	8 a.p.	34½	4,26	8.63	28.00	0.94	1090	94
	1 a.p.	32	4,45	9.71	31.00	1.02	993	96
	Dead	Pressure necrosis with rupture of the uterus Five fetuses approximately one week from term						

moderate normochromic anemia, and in pregnancy No. 25-3 a moderate hypochromic anemia developed. In the last pregnancy, No. 26-3, the blood volume changed very slightly, but a marked decrease in total circulating hemoglobin occurred.

The one pregnancy observed in gastrectomized Dog No. 27 had to be terminated by Porro section, approximately one and one-half weeks from term. Approximately two weeks before term this animal developed a severe hyperchromic anemia and became too weak to stand. After a transfusion of

CONDENSED PROTOCOLS

Gastrectomized Dog No. 26

NO. OF PREG. AND TIME AFTER OPERATION	DAYS ANTE OR POST PARTUM	WT. LBS.	R.B.C. MILLIONS	HB. GM.	HEMATOCRIT	COLOR INDEX	BLOOD VOLUME	TOTAL HB.	MISCELLANEOUS
1st	58 a.p.		6,80	14.00		0.96			Term; 12 pups
2 mo. p.o.	37 a.p.		5,80	12.70		1.02			
	17 a.p.		6,00	11.70		0.91			
	0		5,00	8.65		0.81			
	22 p.p.		6,50	10.30		0.74			
	55 p.p.		7,15	12.50		0.82			
	71 p.p.		7,85	14.20	50.20	0.84			
2nd	54 a.p.		6,50	12.80	45.00	0.92			Term; 3 pups
8 mo. p.o.	19 a.p.		6,40	12.20	38.80	0.89			
	3 a.p.		5,80	11.70	39.00	0.94			
	18 p.p.		6,40	12.05	38.60	0.88			
	91 p.p.		7,23	15.08		0.97			
3rd	55 a.p.	46¼	7,23	15.08	47.40	0.97			Near term; 7
14 mo. p.o.	29 a.p.	46½	7,99	14.03	44.70	0.82	1504	211	pups born
	15 a.p.	42½	6,49	11.82	43.70	0.85	1555	184	dead
	1 a.p.		6,60	11.59		0.82			
	11 p.p.	45	5,83	12.17	40.70	0.97	1405	171	
	48 p.p.		6,72	14.73		1.02			
	98 p.p.	47¼	7,90	15.08	47.30	0.89	1697	256	
Gastrectomized Dog No. 27									
1st	65 a.p.		7,50	11.30		0.70			Transfused
4 mo. p.o.	31 a.p.		8,00	11.90		0.70			with 200 c.c.
	17 a.p.		6,80	11.30		0.78			whole blood.
	0		2,95	8.30		1.31			Porro section
	1 p.p.		4,90	10.70		1.02			—9 pups 1½
	30 p.p.		4,57	9.05		0.92			weeks from
	48 p.p.		3,30	8.00		1.13			term
	50 p.p.		Dead	Diffuse peritonitis					
Gastrectomized Dog No. 33									
1st	57 a.p.	29½	7,65	12.44	36.2	0.76	848	106	6.27 μ Term
5½ mo. p.o.	24 a.p.	33½	6,30	11.19	35.0	0.83	817	91	5.96 μ 10
	0	35½	4,25	6.88	27.0	0.75	892	61	4.36 μ pups
	20 p.p.	30	5,67	9.36	26.5	0.77	908	85	5.94 μ
	44 p.p.		7,06	12.40	35.5	0.76			
2¾ years after gastrectomy No. 33 was subjected to a 6 ft. enterectomy									
Composite of 4 determinations before operation		28	6,80	12.93	46.44	0.89			
2nd	91 a.p.	26½	7,15	14.72	42.50	0.96	1055	155	Term; 3 pups
83 days after	43 a.p.	25¼	6,45	13.14	46.00	0.95	1050	138	
enterectomy	9 a.p.	27¼	4,67	10.50	37.50	1.05	1020	107	
	4 p.p.	23¼	5,15	10.30	33.25	0.93	925	95	
	43 p.p.	26	5,82	12.52	41.50	1.00	1050	132	
	154 p.p.	25½	7,99	16.32	53.50	0.96	930	152	

300 c.c. of blood, the operation was successfully performed, but the animal died fifty days later. Autopsy revealed multiple abscesses of the liver and lungs and a diffuse peritonitis.

Gastrectomized Dog No. 31 was observed during three pregnancies. In pregnancy No. 31-1 a slight hypochromic anemia developed. The blood volume at term was decreased by approximately 300 c.c. and the total circulating hemoglobin by approximately 90 gm. In pregnancy No. 31-2 a marked hypochromic anemia developed. The blood volume at term was decreased by approximately 400 c.c. and the total circulating hemoglobin by approximately

CONDENSED PROTOCOLS

Gastrectomized Dog No. 31

NO. OF PREG. AND TIME AFTER OPERATION	DAYS ANTE OR POST PARTUM	WT. LBS.	R.B.C. MILLIONS	HB. GM.	HEMATOCRIT	COLOR INDEX	BLOOD VOLUME	TOTAL HB.	MISCELLANEOUS
Before gastrectomy		39½	7,60	15.43	56.00	0.95	1540	238	
1st	70 a.p.	42½	8,16	15.10	60.40	0.86			Term; 8 pups
4 mo. 1 wk.	15 a.p.	49	6,95	14.38	39.80	0.97	1296	186	
p.o.	2 a.p.	55	6,33	12.51	35.50	0.92	1240	155	
	17 p.p.		6,07	11.10		0.85			
	79 p.p.	48	9,13	15.78	45.50	0.81	1553	245	
2nd	65 a.p.	Same as 79 p.p. of first pregnancy							
8¾ mo. p.o.	47 a.p.		9,16	16.91		0.86			
	13 a.p.		8,28	13.50		0.76			
	0		5,69	7.52		0.62			
	3 p.p.	41½	5,83	8.15	28.60	0.65	1150	94	Term; 7 pups
	23 p.p.		7,20	10.36		0.67			
	82 p.p.	39½	8,74	12.17	39.60	0.65	1077	131	6.21 μ
3rd	62 a.p.	43	8,11	12.57	32.00	0.72	1125	141	5.94 μ
1¾ yr. p.o.	17 a.p.		8,69	11.23	29.50	0.60			
	6 a.p.	49½	8,19	8.49	27.00	0.48	1470	124	6.04 μ Term;
	1 p.p.		7,36	8.28	24.00	0.53			9 pups
	32 p.p.		9,67	10.20	38.00	0.49			

155 gm. In pregnancy No. 31-3 a marked hypochromic anemia developed. The blood volume at term was increased by approximately 350 c.c., and the total circulating hemoglobin decreased by approximately 20 gm. The mean cell diameter preceding this pregnancy was 6.21 μ. The mean cell diameter at term was 6.04 μ.

Gastrectomized Dog No. 33 was observed during one pregnancy. A severe hypochromic anemia developed. The blood volume at term remained unchanged, but the total circulating hemoglobin was decreased by approximately 44 gm. The mean cell diameter prior to pregnancy was 6.27 μ, at mid-term 5.96 μ, and at term 4.36 μ. Gastrectomized Dog No. 33 was subjected to an enterectomy of six feet of jejunum and ileum. Two weeks after the operation the animal had a moderate hypochromic anemia; but from the thirtieth postoperative day to the one hundred and fourteenth postoperative day, when the animal again became pregnant, the blood picture showed no anemia. During this pregnancy the animal developed a moderate normochromic anemia, the blood volume remained unchanged; but the total circulating hemoglobin decreased by approximately 45 gm.

Normal Dogs.—Each of 12 normal dogs were observed during one pregnancy. The blood picture at term showed a slight normochromic anemia in 5 animals (Nos. 1, 3, 5, 6, and 9), slight hypochromic anemia in one animal (No. 10), moderate normochromic anemia in 3 animals (Nos. 0, 2, and 7), moderate hypochromic anemia in 2 animals (Nos. 8 and 11), and marked normochromic anemia in one animal (No. 4).

In 8 cases in which blood volume determinations were performed a sufficient number of times to be adequate, the blood volume at term was unchanged in 4 cases, Nos. 3, 6, 7, and 10; increased in 3 cases by approximately 175 c.c. in Case No. 0, 300 c.c. in Case 2, and 100 c.c. in Case 5; and decreased in one case, No. 4, by approximately 130 c.c. Of these 8 cases the total circulating

CONDENSED PROTOCOLS

Normal Control Dogs

NO. OF DOG	DAYS ANTE OR POST PARTUM	WT. LBS.	R.B.C. MILLIONS	HB. GM.	HEMATOCRIT	COLOR INDEX	BLOOD VOLUME	TOTAL HB.	MISCELLANEOUS
0	0	28	5,86	12.22	37.00	0.97	980	120	Term
	1 p.p.	23	4,70	10.15	28.50	1.01	755	77	9 pups
	25 p.p.	21¼	4,85	11.61	39.25	1.12	840	97	
	39 p.p.	21	5,05	11.88	38.00	1.10	845	100	
	64 p.p.	22½	7,35	14.93	50.00	0.95	805	120	
1	1 a.p.	37	6,08	12.67	38.75	0.97	1023	130	Term
	2 p.p.	32	5,39	11.78	34.00	1.02	893	105	7 pups
	25 p.p.	24	5,00	10.94	35.00	1.02	822	90	
	Very sick with snuffles. Killed.								
2	5 a.p.	46	4,40	10.20	30.00	1.08	1360	139	
	4 a.p.	46	4,44	10.20	30.50	1.07	1350	138	Term
	2 p.p.	38	4,75	10.25	30.50	1.01	1170	120	8 pups
	26 p.p.	32¾	4,28	9.80	31.00	1.07	1190	117	
	53 p.p.	30½	4,85	11.88	35.00	1.14	1040	123	
3	55 a.p.	17½	6,99	14.40	38.80	0.96	438	63	
	19 a.p.		6,29	14.30		1.06			
	5 a.p.		6,50	14.60		1.05			
	0	18½	6,45	14.20	38.00	1.03	450	64	Term
	11 mo. p.p.	18	6,99	13.47	39.20	0.90	472	64	5 pups
4	9 a.p.	33½	4,28	9.45	28.00	1.03	1110	105	
	6 a.p.	34	4,00	8.89	25.50	1.04	1080	96	Term
	1 p.p.	26	3,56	8.25	25.00	1.08	904	75	8 pups
	93 p.p.	29¼	7,03	12.75	45.00	0.85	1210	154	
5	0	38½	6,08	13.94	44.50	1.07	1190	166	Term
	1 p.p.	32½	4,82	11.00	32.50	1.07	865	95	9 pups
	29 p.p.	31	6,91	14.72	46.50	1.00			
	117 p.p.	26½	7,25	14.41	48.00	0.93	1090	157	
6	1 a.p.	47½	6,57	14.28	45.00	1.01	1370	195	Term
	4 p.p.	38½	5,21	11.48	36.00	1.03	1350	155	8 pups
	38 p.p.	31¾	6,90	12.30	39.50	0.83	1300	160	
	80 p.p.	32	6,65	13.94	45.00	0.98	1360	190	
	96 p.p.	33	7,28	12.98	40.50	0.83	1430	186	
7	19 a.p.	25	5,75	13.49	37.00	1.09	777	105	
	2 a.p.	25	5,91	11.80	34.50	0.93	773	91	Term
	1 p.p.	21	4,60	10.20	29.25	1.03	825	84	7 pups
	6 p.p.	18½	4,21	9.16	28.00	1.01	727	67	
	52 p.p.	21	6,77	13.66	44.00	0.94	743	101	
	55 p.p.	20	6,59	13.06	40.00	0.92	777	102	
8	4 a.p.	49	5,36	12.52	35.75	1.09	1700	213	
	1 a.p.	50	6,40	11.75	37.50	0.86			Term
	1 p.p.	43	5,50	11.24	36.50	0.95	1760	198	5 pups
	24 p.p.	33	4,93	10.10	33.00	0.96	1450	147	
	42 p.p.	Dead. Lobar pneumonia.							
9	6 a.p.	14	6,17	14.72	44.50	1.11			
	4 a.p.	14½	6,39	13.06	42.00	0.96	379	49	Term
	3 p.p.	10¾	5,14	8.75	32.00	0.79			5 pups
10	62 a.p.	21½	7,28	14.73	46.00	0.95	763	113	Term
	41 a.p.	20	7,26	13.75	44.40	0.88	735	101	5 pups
	13 a.p.	23	6,10	10.90	48.20	0.83	784	86	
	5 a.p.		6,54	12.63		0.90			
	2 p.p.		6,01	11.71		0.91			
	18 p.p.		7,09	11.81		0.78			
11	46 a.p.	24¼	7,43	10.62	42.50	0.73	800	85	Term
	22 a.p.	24½	6,72	11.59	43.10	0.80	802	93	6 pups
	8 a.p.		6,42	11.94		0.87			
	1 p.p.	25	6,35	9.43	41.60	0.69	820	77	
	41 p.p.		7,85	13.89		0.83			

hemoglobin at term showed very little change with one exception. In Case 4, which showed approximately 130 c.c. decrease in blood volume at term, the total circulating hemoglobin was decreased by approximately 58 gm.

Summary of Results.—With few exceptions, pregnancy in the gastrectomized dog resulted in a marked or severe anemia. Blood volume determinations made in eight pregnancies demonstrated in every case a total circulating hemoglobin decrease of from 20 to 155 gm. regardless of changes in blood volume. In two gastrectomized dogs subjected to extensive enterectomy, the anemia of pregnancy, as judged from the blood picture alone, appeared to be no more severe than before enterectomy. However, in one case the size of the litter was much less and in the other case dehydration supervened. In both cases the total circulating hemoglobin was markedly reduced.

In general, the severity of the anemia appeared to be related to the size of the litter and to the shortness of the interval between successive pregnancies.

Pregnancy in normal dogs caused a marked anemia in only one case. Hydremia occurred in only 3 out of 8 cases (38 per cent). Anemia of the puerperium was more marked than the anemia of pregnancy in almost every case.

DISCUSSION

The above results indicate that hydremia of pregnancy occurs in approximately one-third of normal dogs and that anemia of pregnancy in normal dogs is uncommon. These findings are in accord with those of earlier authors. Spiegelberg and Gescheidler[12] in 1872 and Nasse[13] in 1876 reported that in dogs the blood was more dilute during the last half of pregnancy. Spiegelberg and Gescheidler[12] did not believe this significant because they found that the percentage of hemoglobin in pregnant and nonpregnant dogs was equal. In 1927, Schmidt and associates[14] reported that a slight hydremia occurred during the last ten days of pregnancy in the dog. They believed that the extent of the hydremia was significant, but they reported the results of only three experiments.

The occurrence of a marked to severe anemia which developed during the last half of pregnancy in the gastrectomized animals appears easily explicable in the light of several reports. Mullenix,[15] Dragstedt,[16] Kellogg,[17] and Mettier[18] and their co-workers have observed that the production capacity for hemoglobin regeneration is markedly reduced in the gastrectomized dog. These authors also found the production capacity for hemoglobin regeneration was markedly increased by the administration of iron. Moreover, Hugounenq[19] reported that in the case of the human fetus two-thirds of the storage of iron takes place during the last three months before birth. Nicholas[20] observed that the storage of hemoglobin in rat fetuses is most rapid during the early part of the last third of the gestation period. And Zeidberg[21] found that in the rabbit the last half of pregnancy is the period during which the fetus is laying down its chief supply of blood-building materials. These facts, along with the fact that anemia was most marked in those gastrectomized dogs bearing the largest litters, lead us to believe that in the presence of a markedly reduced production capacity for hemoglobin regenera-

tion the added strain of fetal demand for blood-building materials uniformly brings about a marked to severe anemia of pregnancy in the gastrectomized dog.

In the light of these experiences let us examine the various hypotheses advanced to explain anemia of pregnancy in women. Strauss and Castle[2] studied gastric secretion during pregnancy and puerperium in 24 women and found that 75 per cent of these women did not secrete normal amounts of free hydrochloric acid and pepsin during more than half of the period of pregnancy. From this and other studies these authors concluded that the hypochromic anemia of pregnancy is due either to a direct dietary deficiency or to a deficiency conditioned by gastric anacidity, hypoacidity, or associated gastrointestinal defects in the presence of fetal demand for blood-building materials. The importance of a gastric secretory defect in the development of hypochromic anemia of pregnancy has been supported by the observations of Rowland,[1] Davies and Shelley,[22] and Goodall and Gottlieb.[23] At first glance, the development of a marked to severe anemia of pregnancy in gastrectomized dogs, but not in normal dogs, seems to lend experimental support to the hypothesis that gastric defects play an important role in the development of a hypochromic anemia of pregnancy. However, Lyon[24] and Fullerton[25] believe that marked hypochromic anemia of pregnancy is probably dependent upon the presence of a pre-existing anemia which was not necessarily dependent upon pregnancy per se. Bethell[26] has gone further to point out that the pregnant woman saves, from the absence of menstrual blood loss over ten periods, from one-third to one-half of the amount of iron utilized by the fetus. He states that the reserves of blood and blood-building materials possessed by the healthy person without a tendency toward anemia are adequate to replace the net iron loss from mother to fetus without demonstrable effect upon the blood level. Bethell concluded from his studies that anemia in pregnancy is commonly due either to pre-existing iron depletion or to an inadequate intake of protein of high biologic value during gestation.

Do these factors play a part in the anemia of pregnancy of the gastrectomized dog? Mettier and Minot[27] have reported that iron is more readily absorbed from an acid than from an alkaline medium. Carrel and co-workers[28] and Emery[29] have shown that digestion and assimilation of protein are markedly impaired in the gastrectomized dog. Moreover, as has been mentioned before, Ivy, Morgan, and Farrell[8] reported the occurrence of spontaneous anemia in the gastrectomized dog. Therefore, the reserve stores of blood-building materials in the gastrectomized dog are, presumably, low. Since normochromic anemia of pregnancy occurred in some gastrectomized dogs (i.e., No. 11-3 and No. 26-2) and in two gastrectomized and partially enterectomized dogs (No. 11-8 and No. 33-2), the factors predisposing to anemia emphasized by Bethell[26] seem especially significant in explaining the anemia of pregnancy in the gastrectomized dog.

Since the achylia gastrica produced by gastrectomy is permanent and leads to a markedly decreased reserve of blood-building materials and to a tendency to spontaneous anemia, the results of the experiments reported here may or may not lend support to the hypothesis that transient gastric secretory defects in pregnant women may cause an anemia of pregnancy. Likewise, from these

experiments, little can be said about the role of the stomach in causing "pernicious anemia" of pregnancy because Richter and associates[30] and Ivy and others[31] have shown that canine stomach is inactive in pernicious anemia. In addition, Bussabarger, Ivy, and Richter[32] have found the entire intestine of the dog to be inactive in pernicious anemia. In view of the fact that the hog duodenum[33, 34] and hog colon[35] have been shown to be active in pernicious anemia, it would seem that too much attention has been focused upon the stomach at the expense of the rest of the gastrointestinal tract.

SUMMARY AND CONCLUSIONS

1. The blood picture of five gastrectomized dogs was observed through a total of fifteen pregnancies. (a) Marked to severe anemia developed in the gastrectomized dogs in eleven pregnancies. (b) Blood volume determinations made in eight pregnancies demonstrated in every case a decrease in total circulating hemoglobin of from 20 to 155 gm., regardless of changes in the blood volume. (c) In two gastrectomized dogs subjected to extensive enterectomy the anemia of pregnancy, as judged by the blood picture alone, appeared to be less severe than before the enterectomy. However, in both cases the total circulating hemoglobin was markedly reduced.

2. The blood picture of twelve normal dogs was observed through twelve pregnancies. (a) Marked anemia of pregnancy developed in only one case. (b) Anemia of the puerperium was more marked than anemia of pregnancy. (c) Blood volume determinations made in eight pregnancies disclosed hydremia in only three cases.

3. The etiology of anemia of pregnancy was discussed.

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TULAREMIA FOLLOWING INJURY WHILE PERFORMING POST-MORTEM EXAMINATION ON HUMAN CASE*

With Report of the Post-Mortem Findings in a Case of Pulmonic Type of Tularemia

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THE sources of infection and the manner of spread of tularemia in man have been summarized by Francis[1] in a recent publication. He lists more than twenty animal and insect hosts capable of conveying the infection, but states that there is no record of transfer of the disease from one human being to another. Having observed an instance of such transfer, we are prompted to report it, and additionally describe the post-mortem findings in a case of the pulmonic type of tularemia.

One of us (E. S. M.) accidentally incised her thumb while performing a post-mortem examination on the body of a negro who, though diagnosed clinically as having had typhoid fever, proved to be a victim of tularemic pneumonia. The development of an ulcer at the site of the accidental wound with associated regional lymphadenopathy suggested the diagnosis of tularemia. This was verified by the subsequent course of the disease and the repeated demonstration in the blood of specific antibodies in titers as high as 1:2560.

CASE REPORT

W. K., a 26-year-old negro, was admitted to the Charity Hospital of Louisiana, New Orleans, on December 15, 1936. He had been in good health until eight days previous to admission, when he experienced a sudden onset of headache and fever, which persisted with increasing severity. Additionally, he suffered recurrent pain in the right lower abdominal quadrant and on two occasions attacks of epistaxis.

One week after his death, the Hospital's Social Service Department learned that he had been a peddler by trade and that seven days prior to the onset of his illness he had skinned two hampers of wild rabbits.

Physical examination revealed an acutely ill adult negro, with a temperature of 105° F., respiration 24, and radial pulse 102 per minute. Blood pressure was 125 systolic and 85 diastolic. The skin was hot, moist, and elastic. There was no palpable lymphadenopathy. Eyes and ears were free of pathology, while the nares were partially obstructed by clotted blood. The lungs expanded equally and with moderate excursion. No abnormalities in the breath or voice sounds were detectable, and no râles were heard. The heart was normal in size, its sounds of good quality and its rhythm regular. There were no murmurs. Tenderness was elicited in the right lower abdominal quadrant, but there was no muscular rigidity. There were no palpable viscera or masses. Examination of the urine was negative. The blood contained 4,000,000 red blood cells and 8,500 white blood cells per c.mm. The differ-

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ential leucocyte count revealed 60 per cent polymorphonuclear neutrophiles, 30 per cent small lymphocytes, and 10 per cent large lymphocytes and monocytes. No malarial parasites were found.

The demonstration in the blood of agglutinins for *Eberthella typhi*, in a titer of 1:320, seemed to corroborate the clinical impression of typhoid fever and treatment for such was instituted. During the ten days he remained in the hospital, his condition grew steadily worse and he died December 25, 1936, at 9 P.M.

POST-MORTEM EXAMINATION

Body: Post-mortem examination was made ten hours after death. Inspection of the body revealed a well-developed, well-nourished, young colored male. Body heat was absent, rigor mortis was present. There were no notable gross lesions or abnormalities.

Peritoneal Cavity: There was no free fluid or adhesions present and the membranes were smooth, moist, and glistening.

Pleural Cavities: No adhesions were present. Both cavities were filled with slightly cloudy straw-colored fluid. The right visceral pleura was covered with patches of yellowish fibrinous exudate and a considerable number of fibrinous strands were free in the fluid. The serosa of the left cavity was free of exudate.

Pericardial Cavity: The cavity contained the usual amount of clear, straw-colored fluid and the serosa was smooth and glistening.

Heart: The heart weighed 350 gm. The myocardium was reddish in color and firm in consistency. The valves presented no abnormalities and the endocardium appeared normal. The coronary arteries were patent.

Lungs: The right lung weighed 1,225 gm., the left lung 900 gm. The serosae of both lungs were grayish pink in color with scattered yellowish areas, measuring 1 to 2 cm. in diameter. Areas of patchy consolidation could be felt throughout all lobes, but the lower right lobe was uniformly firm in consistency. On section there was diffuse consolidation of this latter area with scattered areas of necrosis. All other lobes showed scattered patches of consolidation and necrosis.

Liver: The liver weighed 2,175 gm. The anterior edge was smooth, rounded, and extended 6 cm. below the right costal margin. Glisson's capsule was smooth and normal in color. On section the liver markings proved indistinct, and the surfaces revealed were pale red in color.

Spleen: The spleen weighed 160 gm. It was dark red in color and very soft in consistency. The pulp scraped with ease.

Pancreas, Adrenals, and Gastrointestinal Tract: There were no notable pathologic changes.

Kidneys: The right kidney weighed 210 gm; the left kidney weighed 220 gm. The capsules stripped with ease leaving smooth pink surfaces. On cut section the line of demarcation between cortex and medulla was distinct. The cortical zone was slightly widened.

MICROSCOPIC EXAMINATION

Lung: The sections obtained from the consolidated portions (Fig. 1) showed large areas resembling caseous necrosis with interspersed groups of alveolar sacs filled with exudate. The necrotic areas were composed of either a mixture of polymorphonuclear leucocytes, macrophages, fibrin, and necrotic debris or a granular bluish staining mass of necrotic material in which only a few cellular elements were discernible. In the non-necrotic areas the alveolar septal cells were swollen. The exudate was variable, consisting either of a network of fibrin in which there were scattered polymorphonuclear leucocytes, red blood cells and macrophages, or a mixture of polymorphonuclear leucocytes and macrophages. The exact composition of the exudate in any given place varied between these two extremes. The cytoplasm of the majority of the macrophages was vacuolated or uniformly pale staining (foam cells) but many contained phagocytosed cells or cellular fragments, particularly nuclear fragments. Some of the latter cells measured as much as 20 micra in diameter. No multinucleated giant cells were observed. Although both the arteries and veins in the con-

solidated areas showed polymorphonuclear leucocytic and macrophagic infiltration of their walls, the venous pathology was very much more marked than the arterial. Many veins showed cellular infiltrations, varying from adventitial, medial, and occasionally subintimal accumulations, to diffuse infiltration of their walls. The endothelial cells were swollen and increased in number. Segments of media were necrotic in some, and in a few the lumina were filled with necrotic material. No arterial thromboses were present. The pleura was covered by an exudate of fibrin, polymorphonuclear leucocytes, and a few macrophages.

Heart: The subepicardial tissue was infiltrated by a few lymphocytes and macrophages. Fragmentation of the muscle fibers and interstitial edema were present to a moderate degree. A few polymorphonuclear leucocytes were present in the interstitial tissue in the immediate vicinity of a good number of capillaries, but no diffuse myocardial infiltration was noted. Of greater interest was the perivascular infiltration of cells morphologically resembling Aschoff cells. Except in the adventitia of one artery no nodular accumulations were noted, but groups of four to five cells were observed. Some of these cells had purplish staining cytoplasms with distinct borders and rounded nuclei, but the majority had neutrophilic cytoplasms with indistinct borders and ovoid vesicular nuclei containing a longitudinal chromatin bar.

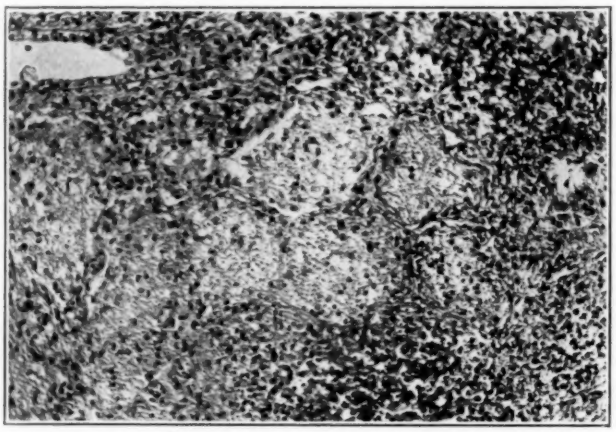

Fig. 1.—Tularemia. Lung, showing exudate in alveoli.

Liver: The liver showed a moderate degree of congestion throughout (Fig. 2). The sinusoidal spaces contained an increased number of polymorphonuclear leucocytes within their lumina. The Kupffer cells were increased in number and size and in almost every high-power field phagocytosis of cells and cellular fragments was observed. The liver cells were swollen, pale staining and showed a slight amount of vacuolization. Many nuclei were swollen, consisting practically of a peripheral ring of chromatin material and a central clear or pale bluish staining area. There were from one to five areas of necrosis in each square centimeter of liver tissue. The largest of these measured approximately 0.5 mm. through its greatest diameter. These foci consisted of rounded or ovoid groups of necrotic liver cells which had been invaded by polymorphonuclear leucocytes. Macrophages in these areas were sparse. Although the foci appeared to be sharply delimited, no appreciable peripheral cellular reaction was present between them and the surrounding liver cells.

Spleen: There was a moderate degree of congestion. The sinusoidal spaces were somewhat dilated and the pulp showed an increase in polymorphonuclear leucocytes. The reticulo-endothelial cells were increased in number and size, many of the larger having vacuolated cytoplasms. An occasional cell had phagocytosed cellular debris. An occasional area of focal necrosis of the pulp was observed similar to those seen in the liver, except that approximately half of them consisted mainly of accumulations of polymorphonuclear leucocytes with little caseous necrosis. One such area of focal necrosis was observed in a Malpighian corpuscle.

The Kidneys, Adrenals, and other Abdominal Organs showed no notable pathologic changes, except a moderate degree of parenchymatous degeneration.

The unusual appearance of the pneumonic process in the right lung impressed one of the authors (E. S. M.) and after injuring her thumb while sectioning this area, she was careful to institute prophylactic treatment. She immediately removed the rubber gloves, washed her hands thoroughly with soap and water, and applied 5 per cent phenol followed by 70 per cent alcohol to the wound. Using a new pair of gloves, she completed the post-mortem examination. Several hours later, as an added precaution, she painted the wound with tincture of iodine. During the next two days there seemed to be satisfactory healing, so the lesion was ignored.

Approximately sixty hours after the injury, she developed headache accompanied by malaise, irritability, and generalized muscular pains. These symptoms increased in severity with exacerbations each evening until that of the fifth day, when she experienced a chill, nausea, and vomiting, and a subsequent rise of temperature to 102° F. On the following morning she entered the hospital, appearing profoundly ill with fever of 102.4°, pulse 90, and respiration 20 per minute. Eyes, ears, nose, and throat were free of abnormalities. The lungs expanded equally, breath sounds were normal, and no adventitious sounds were heard.

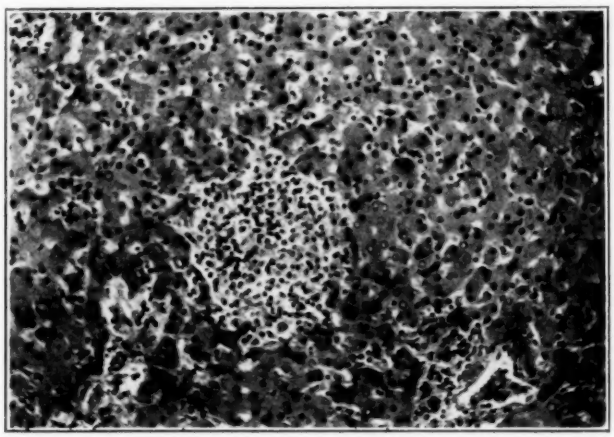

Fig. 2.—Tularemia. Liver, showing focal necrosis.

The heart was normal in size, its rate was increased, its rhythm regular, and there were no murmurs. The blood pressure was 100 systolic and 60 diastolic. At the site of the injury on the dorsal aspect of the left thumb, there was a small partially healed crusted wound surrounded by a narrow zone of purplish discoloration. The left axilla contained a small, firm, tender lymph node but the epitrochlear region was free of adenopathy. Urinalysis was negative. The blood contained 8,200 white cells per c.mm. of which the polymorphonuclear neutrophiles totaled 72 per cent, lymphocytes 22 per cent, monocytes 4 per cent, and eosinophiles 1 per cent. Within forty-eight hours the wound had progressed to definite ulcer formation with shallow, clean floor, elevated edges, and a relatively small inflammatory base. This lesion, accompanied by marked extension of the regional axillary adenopathy and the severe constitutional symptoms, presented the clinical picture of ulceroglandular tularemia. As the disease progressed, headache was persistent and intense, especially in the occipital region. Prostration was marked and the temperature varied from 100° to 104° F., accompanied by recurrent chills with profuse sweating.

On the ninth day of illness the total white blood cell count was 8,500 per c.mm., of which the polymorphonuclear neutrophiles comprised 71 per cent, lymphocytes 25 per cent, monocytes 3 per cent, and eosinophiles 1 per cent. Blood agglutination was positive for *Bact. tularense* in a dilution of 1:160. Two days later an x-ray of the lungs showed bronchopneumonic lesions in the left second interspace and right perihilar regions, although there had not been any cough, dyspnea, or chest pain. The axillary adenopathy had now

increased to the size of a hen's egg, with marked induration and tenderness but without signs of fluctuation. Cultures from the blood were negative but those from the ulcer were positive for *Staphylococcus albus*.

On the sixteenth day the blood agglutination was positive for *Bact. tularense* in a dilution of 1:1280, and during the following week there was marked improvement in all constitutional symptoms, the temperature dropping to a level below 100°. The axillary adenopathy, however, did not decrease in size, but showed evidence of fluctuation and further palpation revealed definite thickening of the ascending brachial lymphatics. An x-ray examination of the chest was repeated and revealed "considerable clearing up" of the bronchopneumonic lesions previously noted.

During the next few weeks there was continued improvement in her general condition with gradual healing of the ulcer and subsidence of the lymphatic involvement. At the end of the seventh week blood agglutination was positive for *Bact. tularense* in a dilution of 1:2560, and the only residual was three small firm lymph nodes in the left axilla.

She resumed her hospital duties on the sixty-second day after the onset of symptoms but has suffered at irregular intervals from attacks of fatigue, malaise, and fever, varying from 99° to as high as 101°. Blood agglutinations were positive for *Bact. tularense* in the eleventh and twenty-eighth weeks in a dilution of 1:320 and on November 20, 1937 (forty-seven weeks after injury), in a dilution of 1:2560. For several days preceding the last determination she had again experienced malaise and fever of 100°, accompanied by tenderness and slight increase in the size of the persistent axillary lymphadenopathy.

An x-ray examination in September, 1937, showed clear lung fields.

COMMENT

The infection here reported was transmitted under circumstances which must be regarded as unusual. Ten hours had elapsed between death and the time of post-mortem examination, the body in the meantime having been kept at temperatures of 34 to 36° F. The site of injury had been immediately cleansed and treated with antiseptics considered of accepted value.

Failure to have established a correct ante-mortem diagnosis in the negro exemplifies the difficulty so frequently encountered in distinguishing clinically between typhoid fever and the typhoidal type of tularemia. This fact, in addition to the lack of characteristic gross pathologic appearances of many of the lesions encountered in tularemia, emphasizes the importance of considering the possibility of the disease in cases of atypical pneumonia seen clinically or in the post-mortem room.

SUMMARY

1. An instance of human to human transmission of tularemia is recorded.

2. The post-mortem findings in a case of the pulmonic type of the disease are described.

3. A case of ulceroglandular tularemia with pulmonary involvement and recovery is reported.

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DIFFERENCE IN GROWTH OF PATHOGENIC FUNGI WITH VARIATION OF MEDIUM AND OXYGEN TENSION*

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PATHOGENIC fungi unlike many bacteria[1] can invade cultural media and grow beneath the surface. Thus, the range of culture media upon which they grow well is increased. The reason for this invasion is probably the fact that oxygen tensions or their equivalent required vary with the medium and with depth of the medium. This paper discusses changes in the site of growth with change in medium and oxygen tension.

The pathogenic fungi and two saprophytes, *Lichtheimia sp.* and *Scopulariopsis brevicaulis*, studied are listed in Table I. They are designated in the body of this paper by the letter following them. Media used were adjusted to pH 5.6. Growths occurred at room temperature in diffused light. Slants were solidified by 1.5 per cent agar. Both glutamic acid and Sabouraud's medium were placed in the same 4 and 6 liter jars. Approximately 50 ml. was allowed each bacteriologic test tube containing about 10 ml. medium.

Anaerobic Conditions Using Chromium Powder-Sulfuric Acid Method.[2]—Two media were used: (a) 0.25 per cent glutamic acid, 4 per cent dextrose, and (b) Sabouraud's medium Difco. Organisms studied were a, h, i, o, p, x, z, aa, bb, ee, ff, hh. A mercury trap was inserted in the apparatus. No growth occurred in twenty-three days.

Anaerobic Conditions in Exhausted Tubes.—The medium used contained 0.25 per cent d-glutamic acid, 4 per cent dextrose. Organisms studied were a, h, i, o, p, x, z, bb, ee, ff, hh. Planted tubes were frozen in liquid air, exhausted until the spark test was negative for oxygen and sealed. No growth occurred in thirty days. Controls frozen for an equal period grew well in the atmosphere.

Growths Under 1 Per Cent Oxygen.—Media, organisms, and results are tabulated in Table I. Jars were exhausted to 38 mm. Hg and filled to atmospheric pressure with nitrogen. Organisms grown on glutamic acid medium were not injured, but many grown on Sabouraud's medium failed to grow in transplant after removal to the atmosphere. Some of these were dry filmlike growths. The media of these organisms supported further growth of transplants from stock culture. Either avidity for oxygen had resulted in separation of these growths from their medium with desiccation, or growth stimulation had been so great that they had "asphyxiated" themselves.

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TABLE I

Amount of growth is indicated on basis of 5 plus or less. The surface growth is indicated above the line and the subsurface below

	S	COS	DOS	EAC	G	COG	DOG
Achorion schoenleinii (a)	4 / s	1 / 2	sp / -	2.5 / -	0.5 / 1.5	vsp / 1.5	1 / 0.5
Acladium castellani (b)	2 / 2	1 / 1.5	vsp / -	0.5 / 0.5	sp / 1	sp / 1	sp / 0.5
Candida candida (c)	2.5 / 2	1.5 / 2.5	1 / sp	3 / 1	vsp / 1.5	vsp / 1.5	sp / 1
Endodermophyton indicum (d)	2.5 / 0.5	vsp / 1	- / -	vsp / 0.5	1 / 1.5	vsp / 1	sp / -
Endodermophyton tropicale (e)	2.5 / sp	sp / 1	- / -	sp / sp	0.5 / 1	- / vsp	0.5 / -
Endomyces capsulatus (f)	2 / 2	sp / 0.75	- / -	sp / sp	vsp / 1	vsp / 0.5	vsp / -
Endomyces dermatitidis (g)	3 / 0.5	0.5 / 2	- / -	- / vsp	1 / 2	vsp / 1.5	0.5 / -
Epidermophyton cruris (h)	5 / 0.5	0.5 / 1.5	sp / -	2.5 / -	2 / 1.5	1 / 2.5	1.5 / -
Epidermophyton inguinale (i)	4 / sp	1 / 2.5	vsp / -	1 / -	1 / 1	1 / 1.5	1.5 / -
Epidermophyton rubrum (j)	4 / sp	0.5 / 0.5	- / -	sp / -	1.5 / 1.5	0.5 / 1.5	2 / -
Glenospora gammeli (k)	3 / 1	1 / 2	- / -	vsp / sp	0.5 / 2.5	- / 0.5	1 / -
Geotrichum bachmann (l)	4.5 / 0.5	1 / 3.5	1 / -	3.5 / -	vsp / 2	- / 2	0.5 / 0.5
Indiella americana (m)	2 / 0.5	1 / 2	vsp / -	2.5 / -	sp / 2	sp / 2	1 / sp
Lichtheimia sp. (n)	4 / 1	3 / 2	4 / -	4.5 / -	2 / 2	- / 1	2.5 / sp
Microsporon audouini (o)	4 / sp	1 / 2.5	sp / -	2.5 / -	0.5 / 2	sp / 1.5	1.5 / vsp
Microsporon felineum (p)	4.5 / sp	1.5 / 2.5	0.5 / -	2.5 / -	1.5 / 1	1 / 2	2 / -
Microsporon gypseum (q)	5 / sp	0.5 / 2.5	1 / -	3 / -	2.5 / 2.5	2.5 / 2.5	2.5 / -
Monosporum apiospermum (r)	4.5 / sp	1 / 2	vsp / -	2.5 / -	sp / 2.5	sp / 2.5	2 / -
Monilia albicans (s)	3 / 1	2 / 2	0.5 / 0.5	3 / 1.5	- / 1.5	- / 1.5	0.5 / 1
Oospora humi (t)	5 / 1	2.5 / 2.5	4 / -	4.5 / -	0.5 / 2.5	- / 3	2 / 0.5
Scopulariopsis brevicaulis (u)	4 / 0.5	2.5 / 2	0.5 / -	3 / vsp	1 / 2.5	vsp / 2.5	1.5 / sp
Sporotrichum schenckii (v)	2 / 0.5	1 / 2	1 / -	1.5 / -	0.5 / 1	- / 1.5	0.5 / vsp
Trichophyton balcaneum (w)	1.5 / 1.5	vsp / 1	- / -	vsp / sp	- / 1	- / 0.5	sp / -
Trichophyton crateriforme (x)	3.5 / sp	0.5 / 1.5	vsp / -	0.5 / -	sp / 1.5	sp / 1.5	1 / vsp
Trichophyton decalvans (y)	2 / 2	- / 0.5	- / -	sp / vsp	vsp / 2	- / 1	0.5 / -

TABLE I—CONT'D

	S	COS	DOS	EAC	G	COG	DOG
Trichophyton granulosum	5	2.5	vsp	3	2	vsp	2.5
	sp	1.5	-	-	2	3	vsp
Trichophyton gypseum (aa)	3	1	0.5	2.5	1.5	vsp	1.5
	sp	3	-	-	1.5	3	vsp
Trichophyton gypseum asteroides (bb)	4	0.5	1	2.5	2	1	3
	sp	2.5	-	-	2	3	sp
Trichophyton gypseum lacticolor (cc)	4	1.5	0.5	3	1	1	3
	0.5	1.5	-	-	2.5	2.5	-
Trichophyton interdigitale (dd)	4.5	2	0.5	3	2.5	1.5	2.5
	vsp	2	-	-	1.5	3	sp
Trichophyton louisianicum (ee)	4	sp	sp	0.5	vsp	0.5	2
	sp	1.5	-	0.5	1.5	1	sp
Trichophyton niveum (ff)	5	2	1.5	2.5	2	1.5	3
	sp	2	-	-	2	3	0.5
Trichophyton purpureum (gg)	4.5	1	vsp	2	0.5	sp	2
	sp	1.5	-	-	2.5	2.5	vsp
Trichophyton sulphureum (hh)	4	1	sp	0.5	0.5	0.5	1.5
	sp	2.5	-	-	1.5	2	-
Willia anomala (ii)	1.5	1.5	0.5	1.5	1	vsp	vsp
	0.5	0.5	0.5	0.5	0.5	1.5	1
Ratio of Growth: $\frac{\text{Surface}}{\text{Subsurface}}$	5.61	0.6	16.1	10.8	0.52	0.23	6.69
Proportionate Amount of Growth	4.0	2.9	0.6	2.0	2.59	2.2	1.56

Designations:
S—Growth, Sabouraud's medium, 17 days, in air
COS—Growth, Sabouraud's medium, 17 days, 99.5 per cent oxygen
DOS—Growth, Sabouraud's medium, 17 days, 1 per cent oxygen
EAC—Growth, Sabouraud's medium, 17 days, enclosed air
G—Growth, glutamic acid medium, 17 days, in air
COG—Growth, glutamic acid medium, 17 days, 99.5 per cent oxygen
DOG—Growth, glutamic acid medium, 17 days, 1 per cent oxygen
sp—sparse growth
vsp—very sparse growth

Growth in a Sealed Atmosphere.—Organisms, media, and results are listed in Table I. Changes are not as marked as with 1 per cent oxygen, and effects on Sabouraud growths not as deleterious.

Growths in 99.5 Per Cent Oxygen.—Media, organisms, and results are listed in Table I. Jars were exhausted five times to 20 mm. Hg and filled each time to atmospheric pressure with oxygen.

Growth Under Increased Pressure.—Media used were (a) 0.25 per cent d-glutamic acid, 4 per cent dextrose, and (b) Sabouraud's medium. Organisms studied were a, i, h, o, p, x, z, bb, ee, ff, hh. The chamber was at a pressure of 50 to 60 pounds the first two days, and at 150 to 160 pounds the next twenty-one days, with the exception of one day when it fell to 100 pounds. The glutamic acid medium showed no growth. On lowering the pressure this medium showed disruption with gas while Sabouraud's medium remained intact. Growths on Sabouraud's medium showed a greater subsurface tendency than in 99.5 per cent oxygen with surface growth almost absent, and less than on glutamic acid medium in 99.5 per cent oxygen.

DISCUSSION

Increased atmospheric pressure and increased percentage of oxygen resulted in growths predominantly subsurface. This was greater in the pressure chamber where the oxygen tension was approximately twice as great. In most instances subsurface growth was good and growth weights would have to be taken to determine the comparative amounts with those of surface growths.

When the surface and subsurface tendencies on the various media and under the various tensions are averaged, that on glutamic acid medium in 99.5 per cent oxygen is comparatively good and has the most subsurface.

When seventeen-day growths are removed from the chambers and growth occurs, it is the type growth that would have occurred if the tubes had been placed in the atmosphere originally.

Growths become more surface in 1 per cent oxygen with no observable decrease in growth on glutamic acid medium, but with marked decrease in growth on Sabouraud's medium where growths appear to be trying to leave the surface and may prove dead. In the latter instance the optimum oxygen tension for growth is absent.

In the atmosphere Sabouraud's medium and 4 per cent peptone, 1 per cent dextrose medium show similar amounts of growth and similar surface tendencies. Media with 0.25 per cent peptone, 4 per cent dextrose, and 1 per cent peptone show about one-tenth the surface tendency and four-fifths the growth of Sabouraud's medium. Other amino acids and hair hydrolyzates show like tendencies to glutamic acid. Pig skin hydrolyzate shows one-half the subsurface tendency of glutamic acid.[3-6] Glutamic acid medium with 0.5 per cent sodium lactate and/or 0.5 per cent sodium phosphate shows about one-half the surface tendency and three-fourths the growth on Sabouraud's medium.[7] The latter effect seems confined to certain organisms.

Factors of importance in growth of organisms may be summarized as follows: Selectivity with reference to the organism is dependent on the atmosphere-medium relationship. The medium is characterized by its constituents, physical and chemical state, and the atmosphere by gas tensions, humidity, temperature, and air velocity. Organisms are characterized by their evolutionary status, adaptations, and resistances. In disease the organism, necessary atmosphere-medium relationship, together with tissue injury, are necessary. Such injury to tissue differentiates pathogenic from saprophytic fungi. In disease selectivity is determined by the constituents of the organ and its oxygen tension. It is questionable that the atmosphere ever functions in a strictly passive sense.

The atmosphere-medium relationship is equally important with bacteria which usually possess no power of invasion of medium and must be studied in shake culture. Increase in oxygen tension alone increases depth of growth, increase in pressure by nitrogen having no such effect.

Skin lesions in guinea pigs at high altitudes due to *Achorion gypseum*[8] have been observed to have a longer incubation period, to be more superficial and less extensive; this has been correlated with clinical findings in tuberculosis. The latter disease is interesting because of extensive work[9] on the gas relations of the tubercle bacillus.

SUMMARY

The atmosphere-medium relationship determines amount, type of growth, and surface or subsurface tendencies of microorganisms.

Invasion of medium results from the organism seeking the most favorable atmosphere-medium relationship for growth and departure of growth. Subsurface tendency apparently bears a proportionality to the anaerobic nature of the organism.

Inhibition of growth claimed by other workers[10] for high oxygen tensions should be decided only after growth weight studies.

Partial oxygen tension determines the depth and presence of growth on a specific medium.

Sparse superficial or no growth on Sabouraud's medium under 1 per cent oxygen often results in death of the organism.

Invasion of tissues and selectivity for tissues in disease are undoubtedly dependent on the medium (tissue)-atmosphere (oxygen tension) relationship and pathogenicity on the added injury through the organism. Simple factors may change this relationship entirely.

Peptone, dextrose, agar from Difco Laboratories, Inc.
d-glutamic acid from Hoffman-LaRoche.
Nitrogen, Airco.
Oxygen, Airco, 99.5 per cent.

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THE PHYSIOLOGIC RESPONSE OF PLEURAL SURFACES TO IMPLANTED DUSTS*

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LATELY several inquiries have sought to establish the physiologic responses of various body tissues to divers dusts in different particle size ranges. As a consequence, there is at hand extensive experimental information dealing with the action of many specific dusts and mixed dusts, some of which have been applied in studies under conditions simulating the exposures experienced by workers in dusty trades. Among others, Mavrogordato, Gardner, and Gye, respectively, have studied the action of dusts in the experimental animal by the inhalation method. In this procedure, it appeared necessary that artificial dusting be continued over a long period, such as from two to five years. Obviously, the time factor hampers inquiries carried out in this manner.

As early as 1924, Sayers,[1] in an effort to reduce the time period required for the appraisal of the properties of unknown dusts, began experiments to determine the action of divers dusts injected into the peritoneal cavity of guinea pigs. He concluded that living animal tissue in any portion of the body tends to respond in essentially the same manner to any dust, but that the action for every individual dust was specific. Selecting the peritoneal cavity as the most suitable point of application, he observed that, in addition to the foreign body reaction provoked by all dusts, highly different changes resulted from the presence of specific dusts. Thus, quartz leads to extensive proliferation of fibrous tissue, but this was not elicited by limestone or coal. In reports based upon continuation of these experiments, Sayers and his associates[2-4] described a technique for the determination of the fibrogenic properties of unknown dusts following deposition within the peritoneal cavity. In addition to the usual foreign body reaction, three general types of specific reaction were described, namely, the proliferative, the absorptive, and the inert. Within ninety days the type of reaction provoked may be determined, and often changes provoked within thirty days may provide proper conclusions.

These three reactions in more detail were found to be:

The proliferative response: characterized by the production of nodules that progressively increased in size. These nodules, when occurring in clumps, fused together and formed a large single mass. Numerous capillaries were present on the surface and throughout the nodules. The appearance was that of cellular proliferation. It was their premise that this pathology was appar-

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ently due to the chemical irritation supplied by the solution of silica in tissue. In ninety days the nodules become more firm, contracted, and fibrous in appearance.

The inert reaction obtained when the injected dust was neither absorbed nor initiated a cellular proliferation. The nodules become more flattened and spreading with time. Fine particles of dust were usually carried over rather extensive areas by phagocytes.

The absorptive response showed the formation of irregular distinct nodules which were often clumped. These nodules became progressively smaller in size as the interval between injection and examination increased, until the original dust eventually disappeared leaving a small area of fine particles at the site of the nodules. In time these also disappeared without the formation of scar tissue.

Thus a reaction was produced in terms of weeks instead of years as was the case in dusting chamber inhalation experiments. The results of these intraperitoneal injection experiments, especially in connection with siliceous dusts, are in conformity with what would be expected from a knowledge of the clinical picture of the actions of these dusts in the pulmonary tissues of human beings.

McCord and his associates,[5-8] in a large series of experiments, supported findings of Miller and Sayers and added two modified types of responses, namely: (1) *a mixed response*, produced by mixed dusts containing some free silica characterized by a reaction which was predominantly inert, but which had a few typical silicotic nodules scattered throughout the tissue, and (2) *a miliary proliferative response*, produced by dusts that possessed more irritant properties than those exhibiting the uncomplicated proliferative reaction. When such dusts were introduced, it appeared that their irritant component incited such marked movement of the organs that the dusts were widely distributed throughout the peritoneal cavity. Wherever particles of this type of dust were deposited, a proliferative response was obtained.

This virtual biologic assay has yielded an abundance of helpful information regarding the harmful effects to be expected from a variety of dusts.

The effect on tissues elsewhere in the body following the introduction of dusts forms the basis for a number of publications. Kettle[9] studied the responses of subcutaneous tissues, and Kettle and Hilton[10] introduced dusts directly into the lungs through the trachea. Their conclusion from this latter study was that the reaction of the lung to dust introduced in this method is essentially the same as to dust introduced by the inhalation method and may advantageously replace the dusting chamber for many purposes for which it has hitherto been considered necessary. Policard[11, 12] used the cornea and conjunctiva in his studies. Drinker and his associates[13] and Stuber[14] have injected sericite and industrial dusts containing free silica into the lymphatics of dogs. It was their opinion that the cellular response of the draining lymph nodes might be developed into a rapid means of determining the toxicity of certain dusts.

It is not known that any of the aforementioned reports embrace observations with reference to the pleural cavity as a site for the experimental implantation of dusts. The creation of typical characteristic reactions at this site would serve a good purpose, offsetting the unfavorable criticism that the responses obtained from such other tissues as the peritoneum, subcutaneous areas, etc., might not reflect the change produced in pulmonary tissues. Therefore, it has been the objective of this present work to make inquiry as to the responses of pleural tissues in the presence of implanted dusts.

The introduction of materials intrapleurally is rather common, especially in the use of oils in connection with pneumothorax. In the field of inorganic materials, Higgins and Lemon[15] injected finely divided particulate graphite into the pleural cavity, using dogs as the experimental animal. During the first few hours after injection, a black, serous, cellular exudate was found in which polymorphonuclear leucocytes were predominant, while lymphocytes and monocytes were present in a small per cent only. However, after seventy-two hours, the picture was reversed with the mononuclear cells present in great abundance and polymorphonuclear cells in lesser abundance. The mediastinum and pulmonary ligaments were the most actively absorptive structures of the thorax, whereas the pericardial, diaphragmatic, and parietal pleura were relatively free of pigment. The lungs invariably were free of pigment, only the lymphatic system being involved in removing cells and free graphite from the mediastinum. Polymorphonuclear cells disintegrated without any further transformation. Merklen, Waitz, and Kabaker[16] injected India ink and trypan blue into the pleural cavity and found that in general they were phagocytized by vacuolate and endothelial cells and monocytes. Arnaud, Chadourne and Weiller[17] injected 10 per cent creosote in alcohol as an irrigation measure in tuberculosis and produced a strong fibrinous reaction. Desbaillet[18] dealt with the reaction of the pleura of the guinea pig to tar and to olive oil. Evolving tissue modifications showed that the pleura hypertrophied, acquired an apical brush, and devoured foreign particles. There occurred proliferation and embryonic tissue formation with verrucosities, villosities, or more often fungoid masses with a contorted surface. After absorption, the pulmonary parenchyma showed simple hypertrophy of alveolar epithelium and proliferation with an adenomatous aspect. Tar emulsion was split by the pleura, the oil being absorbed and the tar caught up by tissue proliferation or devoured by large mononuclears. Bratiano[19] injected animal fats into the pleural cavity. Matsuda,[20] in studies of absorption and elimination by serous membranes, found that the absorption of organic pigments, acid and basic, showed the peritoneum most active, the pleura less active, and the pericardium least active. Brodsky[21] studied the rate of absorption of potassium ferrocyanide from the pleural cavity of dogs and showed that the resorption of the crystalline substance occurs through the lymphatics to the mediastinal lymph nodes and thence into the blood stream without passing through the lung tissue. Bethune[22] believes that selective pleural adhesions, without fluid formation, can be produced as a preliminary to surgical operations by using a fine commercial talc powder which is blown into the pleural cavity, thus dusting the exterior of the lung.

THE PRESENT WORK

In the present series of experiments 50 rabbits were used. They received varying doses from 1 to 4 c.c. of a dust suspension (1 c.c. of suspension equals 0.1 gm. dust). Ten received amorphous silica;* ten, white rouge;† ten, calcium carbonate;‡ and ten received magnetite.§ The remainder were not injected and used as control animals. The majority of animals were submitted to autopsy thirty days after injection. Twenty animals were autopsied, as a control measure, at periods of fifteen, forty-five, sixty and ninety days. The injected material containing as much as 0.4 gm. of dust did not result in any apparent discomfort to the animals either immediately after injection or for the duration of the experiment. The weights of the animals over the experimental period showed normal gains and infection or injury produced no loss of animals.

Preparation of the Dusts.—Four dusts, shown by intraperitoneal injections into guinea pigs[4, 8] to be representative of the three main types of reactions elicited by dusts in animal bodies, were chosen for this experiment: white rouge and amorphous silica as proliferative dusts, magnetite as an inert dust, and calcium carbonate as an absorptive dust.

The dusts were injected in the form of suspensions, prepared by placing 10 gm. of dust in 100 c.c. of isotonic saline solution and shaking thoroughly.

Technique of the Injection.—No preliminary preparation of the animal was found necessary. The rabbit was stretched out by his front and rear legs on a table with the side to be injected uppermost. A 5 c.c. hypodermic syringe with a glass plunger and an 18 gauge needle were used to inject the suspensions. With particles of the size used, little difficulty was experienced due to locking of the syringe.

The pleural cavity was entered by introducing the needle through the skin in the fourth intercostal space about 3 cm. lateral to the sternum, directing the needle downward and slightly backward and carefully inserting the point until it barely penetrated the parietal pleura and entered the free pleural space. When certain that the needle was in the free space and not in the deep muscles or chest organs, the suspension of dust was injected. As a precaution against the possibility of entering the lung tissue, it was found advantageous to introduce a few cubic centimeters of air into the pleural space before injecting the material, thereby producing a partial pneumothorax.

*Amorphous silica—an air floated cryptocrystalline material, specific gravity 2.6, 100 per cent through 600 mesh. Contains 98.47 per cent free silica (SiO_2). Purchased from Fredrick B. Stevens Co., Detroit. Size distribution of particles—25 per cent under 1 micron, 75 per cent under 2 microns, 95 per cent under 5 microns, 99 per cent under 10 microns.

†White rouge—air floated from "Silica #00" mined in Southern Illinois. Contains 99 per cent free silica (SiO_2), 100 per cent through 600 mesh. Purchased from Terminal Sales Corp., Detroit. Size distribution of particles—15 per cent under 1 micron, 62 per cent under 2 microns, 86 per cent under 5 microns, 96 per cent under 10 microns.

‡Calcium carbonate—a Mallinckrodt analytical reagent, more than 99 per cent calcium carbonate, mechanically screened through a 325 mesh screen. Size distribution of particles—10 per cent under 1 micron, 20 per cent under 2 microns, 46 per cent under 5 microns, 77 per cent under 10 microns.

§Magnetite or black iron oxide (ferrous-ferric)—a Mallinckrodt reagent mechanically screened through a 325 mesh screen. Size distribution of particles—25 per cent under 1 micron, 55 per cent under 2 microns, 75 per cent under 5 microns, 87 per cent under 10 microns.

Gross Pathology.—White rouge and amorphous silica, composed almost completely of free silica, produced identical gross reactions in the pleural cavity of the rabbit. The siliceous material was scattered throughout the cavity, although the larger deposits were found at the site of injection on the chest wall, and on the pericardium. Numerous small deposits were found on the lungs and on the mediastinal pleura overlying the vena cava. Nodules were rarely found on the diaphragm, which is worthy of note since in intraperitoneal injections the diaphragm is one of the tissues more frequently affected. This picture of the distribution of dust in the pleural space was true for black iron oxide and calcium carbonate as well as for the silica dusts.

White rouge and amorphous silica (Fig. 1) produced nodules that were comparatively large in size and showed a strong tendency to fuse with those adjacent. Deposits as large as 2 cm. in diameter, adhering to the pleura and

Fig. 1.—Free silica (white rouge). Large irregular masses on pleural surface of chest wall characteristic of a proliferative reaction.

pericardium, were commonly seen after thirty days. Numerous capillaries were present on the surfaces and throughout the nodules, giving the appearance of cellular proliferation. In almost every case, thirty or more days after injection, numerous adhesions between chest wall and lung, chest wall and pericardium, pericardium and lung, and lung and diaphragm were observed.

Calcium carbonate produced a reaction in the rabbit chest that has been designated as absorptive. Forty-five days after injection the material had completely disappeared from the body and to all appearances had left the chest uninjured. After thirty days, two of the rabbits showed a single, small, round, hard, white deposit of calcium carbonate loosely adhering to the pleural surfaces by one or two fine strands of fatty connective tissue. Capillaries were not present in or on the deposits and there were no evidences of cellular proliferation.

Magnetite (Fig. 2) tended to aggregate into one large flat smooth glistening deposit, either at the site of injection or on the pericardium. Occasional small deposits were seen on the visceral pleura. Ninety days after injection the quantity of magnetite in the pleural cavity approximated the amount injected. No cellular proliferation was observed. Thus, since neither a diminution of material nor cellular proliferation was in evidence, the reaction was termed inert.

Whenever animals were dispatched during early days after the injection of any dust, there were observable the usual and expected evidences of a foreign body reaction. Prior to the expiration of thirty days, the evidences of the initial foreign body reaction had completely subsided.

Fig. 2.—Black iron oxide (magnetite). Masses of pigmented material on pericardial pleura. An inert reaction.

Microscopic Pathology.—White rouge (Fig. 3). Microscopically the tissue response is the proliferative reaction characteristic of free silica. There are large masses of cells having the appearance of macrophages in which can be seen particulate foreign material. In many areas the nuclei are more or less distinctly grouped in rosettes around collections of particles of foreign material. In many of these nuclear groups the boundaries between the nuclei are indistinct or not visible, giving these masses the appearance of aggregation giant cells. In a few areas two types of change are observed in the nuclear rosettes. One change consists of an intensification of staining, an increase in opacity of the cytoplasmic material, and a decrease in cytoplasm relative to the amount of nuclear material present. The other change ob-

served consists of an increase in the cytoplasm in proportion to the amount of the nuclear material with the cytoplasm becoming paler. Here the nuclei are small and are placed in the extreme periphery of the cell mass or aggregate. In a few cell groups where these cell processes are most marked, a nuclear fragmentation occurs. These changes seem to represent progressive stages of degeneration. Fibroblasts and young fibrous tissue cells are present in varying numbers in different sections of the material and also in different portions of the same section.

Magnetite (Fig. 4). Microscopically the tissue response is the so-called inert reaction. Here large amounts of deeply pigmented particulate material appear in the sections. Most of this occurs intracellularly and has the ap-

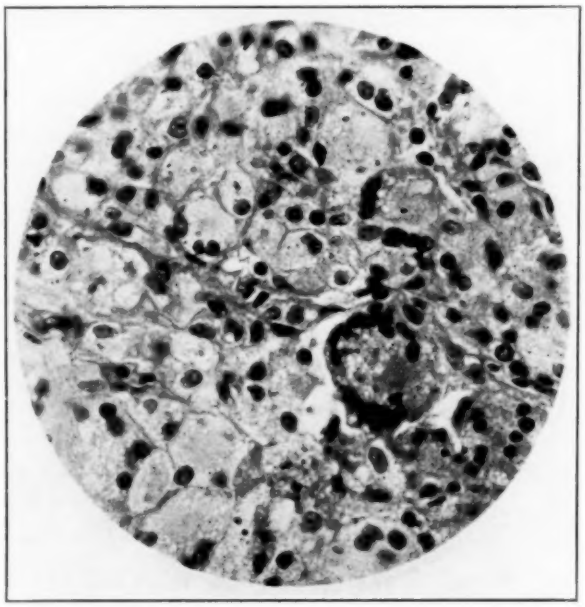

Fig. 3.—Free silica (white rouge). Photomicrograph (high power). Section from nodule shown in Fig. 1. A proliferative lesion with giant cell formation and particulate foreign material in surrounding cells.

pearance of being phagocytized in such large quantities that the details of the phagocytic cells are obscured. A delicate connective tissue meshwork with a few fine capillaries supports the phagocytic cells. There are also present a few large angular masses of foreign material and around these are typical foreign body giant cells.

Calcium carbonate. Microscopically no characteristic changes are apparent.

Multiple Injections in the Same Animal.—It was carefully noted that in no case was there a spread of material from the injected side of the chest to either the opposite pleural space or to the peritoneal cavity. This suggested, in the interest of economy, the utilization of one animal for the testing of three dusts simultaneously, injections being made into each pleural cavity and into the peritoneal cavity.

A second way in which the three body cavities of one animal may be employed is to use a single dust but to make the three injections at such intervals that at autopsy it will be possible to observe the resultant pathology fifteen, thirty, and sixty days after injection.

In this study ten of the experimental animals were submitted to the implantation of dusts into both pleural spaces, and in one case dust was introduced into all three of the sites suggested above. In no case did the animals show any ill effects from the multiple injections.

X-Rays.—X-rays were made of the chests of five rabbits to determine whether the characteristic proliferative nodule produced by free silica could be distinguished from nonproliferative nodules by this means. Two of the five rabbits had received white rouge, two amorphous silica, and one magnetite.

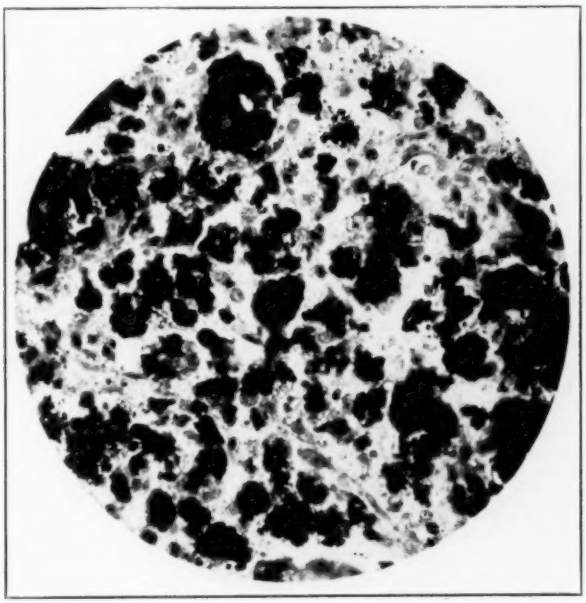

Fig. 4.—Black iron oxide (magnetite). Photomicrograph (high power). Section from pigmented mass shown in Fig. 2. An inert reaction.

Definite shadows could be seen on the x-ray plates of the magnetite rabbit and on one of the white rouge rabbits. The location of these shadows was subsequently correlated with the autopsy findings, but it was concluded that although the nodules could be readily seen on the x-ray plate, they were of no diagnostic significance.

SUMMARY

Forty rabbits were subjected to intrapleural implantation of dust and, with ten additional rabbits as controls, were autopsied after fifteen, thirty, forty-five, sixty and ninety days.

Typical inert, absorptive, and proliferative reactions were produced by the pleura in response to various dusts.

These responses were essentially the same as produced in serous cavities elsewhere in the body by other workers.

The injection of a dust or several dusts into both pleural cavities and into the peritoneal cavity of the same animal was found to be a practical procedure resulting in the saving of animals and time.

The pleural cavity, due to its close proximity to lung tissue, proved to be a suitable location for the study of the physiologic response of serous surfaces to implanted dusts.

The authors extend thanks to W. L. Brosius, M.D., Pathologist, Department of Health, Detroit, for microscopic examinations of tissues and preparation of statements in this report dealing with microscopic changes.

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SEASONAL AND REGIONAL FACTORS IN ACUTE RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE*

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MEDICAL science has made little headway in its attack on the problems of rheumatic infections and the more chronic forms of arthritis, although the recent increase in investigative interest gives rise to a degree of hope. These ailments probably rank close after respiratory infections as disabling factors in the productive activities of human existence. And they have been just as resistant as has the common cold to investigative success. There is, in fact, a more significant relationship between rheumatic infections and those of the upper respiratory tract than this similarity in resistance to investigative success. A great many of the initial rheumatic fever attacks are actually ushered in by acute upper respiratory infection, and both show the same seasonal relationship to storminess. Close study by a number of investigators, however, has failed to provide conclusive proof that the respiratory infections are direct exciting causes of the rheumatic attacks, even though the two may often be closely associated in their seasonal and regional timing.

It is well, therefore, to look closely into all possible factors that may produce regional or time differences in the disease incidence or severity, in the hope that useful etiologic or therapeutic suggestions may show forth. Hence, in this article will be set forth certain significant relationships in the regional and time distribution of rheumatic afflictions. The evidence presented indicates that some factor concerned with the movement of major storm areas over the earth's surface exerts a dominant influence in determining the incidence of these attacks. Let us look briefly at this evidence.

Rheumatic infections, and the resulting disabilities, are perhaps more directly and completely conditioned by climatic and weather characteristics than are any other diseased states. Complaints of this type are infrequent among residents of the tropics or sub-tropics, and increase rather steadily as one passes outward into the temperate zone storm belts. Rheumatic fever attacks and rheumatic heart disease occur ten to twenty times more often in the worst areas of the storm zones than in the calm warmth of the tropics. And in northern countries their difference in incidence between winter high and summer low is almost as striking as is the regional one. At Cincinnati acute rheumatic fever attacks are over six times as frequent in March as in August and September, while at Buenos Aires little seasonal variation attends the more equitable year-round climate. Then, too, those afflicted in the north usually show improvement in symptoms on migration to the climatic calm and warmth of subtropical regions.

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In Fig. 1 is presented the seasonal distribution of acute rheumatic fever attacks in three widely separated hospitals, one in Cincinnati, another in San Francisco,[1] and the third in Peiping, China.[2] Each curve shows the marked increase in attacks that comes with winter cold and storms, and the great diminution in summer warmth. The accompanying curve of temperature variability due to the passage of storm areas over northern United States is seen to parallel the monthly incidence of rheumatic attacks at Cincinnati quite closely, just as we found for the onset of symptoms of pulmonary tuberculosis at this same city. At Peiping the difference in incidence between winter and summer is slightly less marked than at Cincinnati, and at San Francisco the winter peak is only four times the summer low, while at Cincinnati the attacks are six and four-tenths times more prevalent in late winter. This lesser seasonal swing at San Francisco may well be causally related to the lower incidence of winter storm

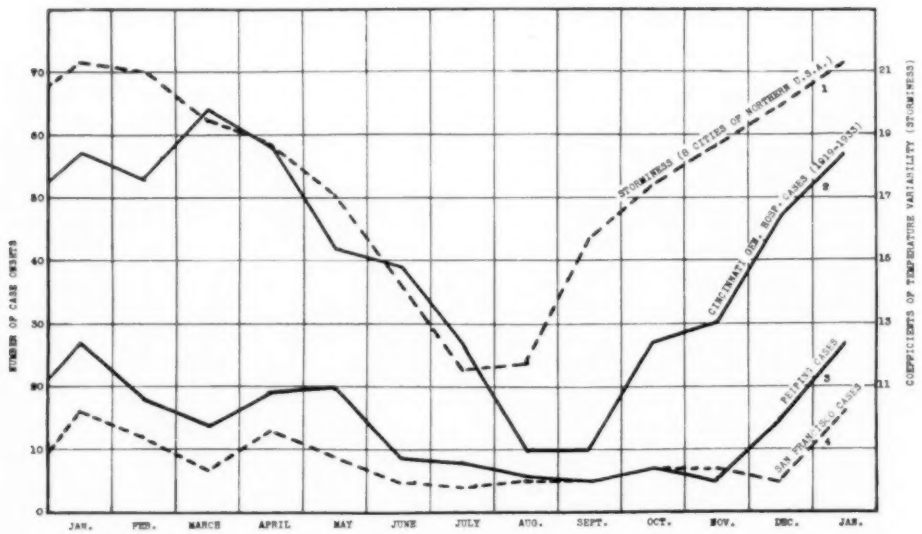
NUMBER OF CASE ONSETS
COEFFICIENTS OF TEMPERATURE VARIABILITY (STORMINESS)
STORMINESS (6 CITIES OF NORTHERN U.S.A.)
CINCINNATI CITY HOSP. CASES (1910-1933)
PEIPING CASES
SAN FRANCISCO CASES
JAN. FEB. MARCH APRIL MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN.

Fig. 1.—Seasonal fluctuation in frequency of acute rheumatic fever attacks.

fluctuations there, for winter and spring weather at Cincinnati is much more subject to abrupt and marked change than is that of San Francisco or a similar latitude in North China.

Regional differences in the incidence of *rheumatic heart disease* have been best estimated by careful examinations of groups of school children. Nichol[3] found only one-fourth as much such heart disease among 1,500 native school children of Miami, Fla., as in a similar number who had migrated to Miami from northern states. Even more marked differences were found by Paul[4] on examination of Indian school children, as shown by the data below:

LOCATION OF GROUPS	LATITUDE	NUMBER EXAMINED	PER CENT WITH RHEUMATIC HEART DISEASE
Montana and Wyoming	44-46°	688	4.5
Northern Arizona and New Mexico	36-37°	1,106	1.9
Southern Arizona	32-33°	1,019	0.5

The same investigator[5] found a 2.2 per cent incidence among 2,624 school children in and around New Haven, Conn., at a latitude between that of the northern and middle groups of Indian children.

Among hospitalized populations there is evident an even greater difference in incidence of *rheumatic heart disease*, estimates of its prevalence in the south ranging from one-twentieth to one-tenth that found in northern hospitals.[3] Faulkner and White[6] showed that this finding had world-wide application, for hospitals of middle temperate regions usually had ten to twenty times as great a percentage of their patients so afflicted as was to be found in hospitals of the tropics or subtropics. Coburn[7] found very little rheumatic involvement in Puerto Rico or other tropical countries, although Puerto Ricans migrating to New York had a relatively high frequency of rheumatic infections. There would

Fig. 2.—Regional variations in acute rheumatic fever death rate.

seem, then, to be little doubt that rheumatic heart involvement is decidedly more common in the cooler, more stormy portions of the temperature zone than in tropical or subtropical warmth.

Similar differences in the incidence of *acute rheumatic fever* have been found among hospital populations north and south,[6] although no survey of incidence has yet been made in nonhospitalized populations. In Fig. 2 is presented the distribution of acute rheumatic fever *deaths* per 100,000 population (white race only) for a five-year period (1926-1930), showing a much less striking difference in *deaths* from the disease than in its *incidence* among the living. The death rate in the two worst areas is only about twice as high as it is in the least afflicted regions. The Rocky Mountain and higher plateau states to the east form one area of high death rates from this cause, while from Iowa east-

ward to the Atlantic Coast is located a second unfavorable region. A decided latitude gradient is also in evidence in the figures for the white population as given in Fig. 2, but negro death rates afford more striking evidence in this respect.

ACUTE RHEUMATIC FEVER DEATH RATES PER 100,000 COLORED POPULATION (1926-1930)

Maryland	7.73	Kentucky	8.31
Virginia	6.39	Tennessee	5.23
North Carolina	4.53	Alabama	3.74
South Carolina	4.25	Arkansas	2.87
Georgia	3.73	Oklahoma	2.76
Florida	3.23	Mississippi	2.54
		Louisiana	2.19

Negro rates are in every state higher than those for the white population, and the increase northward is also more rapid, as has been found to hold with many other climatically conditioned diseases. The latitude gradient is quite evident, however, with both white and colored populations. It can scarcely be related to sunlight intensity, because of the relatively low death rates found well upward along the Pacific Coast and in part of New England. Even in the belt of lower rates up the Plains states the northward increase is seen. No explanation can be offered for the lower death rate in the Plains states, but in this connection it is worthy of note that Faulkner and White[6] cited a remarkably low incidence of the disease and its heart complications in hospital populations of Omaha. This city, the only one cited in the Plains region, recorded a hospital incidence far below that of other cities of similar latitudes.

The reason for the general discrepancy between acute rheumatic fever *deaths* and the *incidence* of the disease and its heart complications in living populations is probably to be found in a more vigorous body resistance to the infection and higher survival rate in the more energetic northerners. This difference in ability to fight such infections as acute appendicitis and tuberculosis has been pointed out[8] as probably due to the generally higher metabolic level and higher vitality of people living in the northern states, as compared to those living nearer the Gulf of Mexico. Such a difference explains quite satisfactorily the finding of a smaller increase in acute rheumatic fever *deaths* toward the north than is found for the *incidence* of the disease in school children and hospital populations. Deaths increase approximately twofold in the worst regions as compared to the regions of low rates, while a tenfold difference in incidence is found. Ability to fight the infection must, therefore, be several times higher in the north than in the south, just as was demonstrated for acute appendicitis and tuberculosis.

In further support of these differences in the disease between our northern and southern states is a seasonal difference described by Greenwood and Thompson[9] for London, Young[10] for England and Wales, Christie[1] for San Francisco, Dieuaide and Chang[2] for Peiping, and Faulkner and White[6] for eastern United States. In Fig. 1 have been set forth the seasonal variations in acute rheumatic

fever attacks at Cincinnati, San Francisco, and Peiping, China. Coburn[7] describes a somewhat similar seasonal distribution of acute rheumatic admissions to northern hospitals. He states that at Buenos Aires little seasonal difference in attack frequency exists, winter being only slightly higher than summer. This again points to weather instability as a dominant factor, particularly when associated with cold or body chilling from exposure. Storminess at Buenos Aires is greatest in their summer season, the winters being cool but calm. Swift[11] also cites several studies relating the frequency of attacks quite closely to periods or seasons of changeable weather.

Based on these definite regional and seasonal differences in the disease, various groups of rheumatic heart disease patients have been sent from northern regions to tropical warmth,[7, 12] and with good results. While in the calm warmth of Miami or the West Indies, northern-born patients experience fewer recrudescences and show improvement generally. Such improvement is soon lost, however, when they must again experience the northern winter and early spring weather. For lasting benefit they must forego living in the stormy north and take up permanent residence in subtropical warmth. Due to the rarity of storm changes in the Southwest, residence there near the Mexican border would probably be found even more beneficial than in southern Florida or the West Indies, where storms of the tropical hurricane type so frequently come. There is a strong suggestion that the sudden change of barometric pressure, which accompanies tropical hurricanes, may be just as important a factor in promoting many types of infectious attacks as are similar pressure changes accompanying temperate zone storms. In temperate regions we have been prone to place more blame on temperature and humidity fluctuations in explaining the evil effects of sudden storm changes, but further research may well show pressure change to be another responsible factor. Rentschler and co-workers[13] found closer correlation of arthritic pain attacks with storm changes in barometric pressure than with other meteorologic factors.

Mention should be made of the severity of acute rheumatic infections in one subtropical region of relatively low storminess. In the mining region of South Africa rheumatic infections seem to be as severe as in any of the stormy temperate climates. Whether this severity is related to work in the deep mines cannot be said until the question has been closely investigated. Very high respiratory disease death rates were observed among these miners until air conditioning in the mines lessened the temperature differences between their depths and the outside air (and, of course, also aided in cleansing the air in the mines). Close study of the rheumatic problem in the South African region might provide information of great value in regard to the general biology of these afflictions.

The more even seasonal distribution of rheumatic fever attacks between summer and winter in Buenos Aires, reported by Coburn,[7] is of some significance in establishing the etiologic importance of atmospheric instability, especially when such storminess is associated with lower temperatures. Abrupt temperature changes seldom strike southern hemisphere inhabitants during the winter season. In the north, on the other hand, we not only have frequent and abrupt

cold waves, but they usually are ushered in with precipitation to enhance the chances for body chilling. Young[10] showed for England and Wales that rheumatic fever attacks bore a definite relationship to precipitation. He also found, as did Paul and associates[5] that the attacks were most frequent among the poor, *who are likely to have least effective protection against weather changes in their dwellings or clothing* (italics and suggestion mine). A critical observer cannot but be impressed by the frequency with which hospital histories record body wetting or chilling just preceding the rheumatic attack. The frequency of acute respiratory infections also seems to play a definite part in the initiation of the rheumatic attacks, but they too are initiated largely by weather changes. By and large, then, we must consider rheumatic fever and its complications directly or indirectly conditioned by the climate and the weather. Proper protection from sudden weather changes should go far to reduce the likelihood of rheumatic attacks.

But for the individual already afflicted, such protection is usually inadequate and migration out of the storm belts is advised. Where economic status permits, those afflicted with rheumatic heart disease (or chorea) should be taken south to avoid the cold and storminess of the winter and early spring season. If possible, permanent change of residence is still more preferable, *the choice region from a climatic standpoint in this country being the southern parts of New Mexico, Arizona, and California.* For the great number of afflicted children whose parents cannot afford such a change, a good suggestion would be that convalescent camps or farms be established by the government in choice spots of the Southwest where the best possible chance for recovery, or an existence of limited usefulness, may be allowed. The total burden on public funds would probably be no more than is required by their frequent and prolonged stays in northern hospitals at high overhead costs. Such convalescent units in the Southwest could well serve for patients from the North with a variety of afflictions (chronic sinusitis, chronic bronchitis, arthritis, and tuberculosis) as well as the rheumatic heart patients. Those suffering from overstimulation or metabolic exhaustion in the North (patients with diabetes, pernicious anemia, hyperthyroidism and early hypertensive tendencies, or heart failure) would also do much better by similar change of residence, although their region of first choice should perhaps be in the moist heat of the Gulf states.

Since the disabling character of rheumatic afflictions in the North makes the care of such patients a heavy burden on their families or public institutions, it would really seem a wise public health move for the federal government to sponsor these health camps. Such a move would involve recognition of a new principle in public health activities, since the presence of these diseases in a population group entails no known health threat to persons not afflicted, with no call for segregation. But why should not public attention be given to the care of the afflicted themselves? The indicated treatment being change of residence to a distant state, sponsorship of such a program must devolve on federal government agencies. Many details of organization and operation of this move-

ment would need careful study, but the obvious benefit to the mass of afflicted individuals warrants the effort.

In recent years considerable numbers of northern rheumatic patients have been sent to Florida for successive winter seasons, and with rather disappointing results. Recrudescences have occurred during such southern sojourns, and the general course of the disease has not been markedly altered in many cases. On the basis of storminess, or weather variability, this result is rather to be expected. Winter in Florida has almost as great a degree of weather variability as does northern winter, although at a much higher mean temperature level. One should not expect, then, much help to rheumatic patients in a southern sojourn for the winter months only. The stagnant summer warmth is the more beneficial time. The Southwest, on the other hand, shares very little in the winter storminess that afflicts the remainder of the country. The following data illustrate well the futility of migration to Florida for the winter months alone, in an effort to escape storm effects:

COEFFICIENTS OF TEMPERATURE VARIABILITY

STATIONS USED	JAN.	FEB.	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
Boston, New York, Cincinnati, St. Louis, Chicago, Minneapolis, Cleveland, 1871-1932	21.3	21.0	19.5	18.7	17.1	14.3	11.5	11.8	15.7	17.4	18.7	19.9
Tampa, Raleigh, Houston, El Paso, 1926-1928	19.9	14.6	17.0	15.7	12.0	11.1	7.6	5.3	11.0	14.4	14.7	18.2

In summary, then, it seems safe to say that in acute rheumatic fever we have an infection much similar to those of the respiratory tract in its climatic conditioning. It strikes most frequently in the cooler, more stormy portions of the temperate zones, and is there a more violent form of infection. In the tropics and subtropics it is markedly less frequent and more benign in course. The survival rate, judging roughly from the data at hand, seems to be considerably higher in the more vigorous people of the north, just as has also been found true in tuberculosis and acute appendicitis attacks. Acute rheumatic fever strikes most frequently in winter and early spring months and seldom in summer warmth. Children with rheumatic heart disease also do best in summer warmth and suffer more recrudescences during winter and early spring. Since the heart damage is permanent and recrudescence so common in northern cold and storms, it is definitely indicated that these unfortunate patients be taken to a subtropical climate where the calm warmth will allow the disease to take a more benign course, and where the lowered general metabolic level will lessen the work load on the damaged heart muscle. Migration for such patients should be to regions well south, preferably near the Mexican border from El Paso west, rather than to southern Florida or the West Indies where tropical storms and long debilitating summer heat might mitigate against chances for complete quiescence of the disease process. Migration should be permanent, and not for the winter alone.

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REPORT OF A CASE OF ESSENTIAL HYPERTENSION OF MORE THAN TWENTY-FIVE YEARS' DURATION SHOWING NO RENAL ARTERIOLAR CHANGES AT AUTOPSY*

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THE question still remains: Does the renal arteriolar sclerosis of essential hypertension, which is said to be a constant accompaniment of this disease, precede the rise in vascular tension?

Experimentally it has been demonstrated that renal ischemia causes hypertension. On this basis there is the possibility that in essential hypertension renal arteriolar sclerosis impairs the blood supply to the kidneys, giving rise to a state of chronic ischemia which in turn induces the sustained elevation in the blood pressure.

Goldblatt[1] and his co-workers have demonstrated that by constricting the renal artery they have been able to produce hypertension. They showed that a state of renal ischemia resulted and that in response to this a pressor substance was liberated by the kidney and poured into the circulation through the renal vein. These findings have been corroborated and have enjoyed general acceptance.

*From the medical services of the Hospital for Joint Diseases, Dr. A. A. Epstein, Chief, New York.

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However, the application of these experimental findings to cases of essential hypertension requires further elucidation. The hypothesis already mentioned assumes that the arteriolar sclerosis is the initial disturbance which causes the augmented blood pressure.

The purpose of this paper is to report an illustrative case pertinent to this question. The patient was a known hypertensive for more than twenty-five years, and at the autopsy showed normal kidneys and normal arterioles.

CASE HISTORY

F. R., first consulted me in December, 1928. She was then 43 years old. When she was 22 years of age, it was discovered that she had high blood pressure. When she was 25, she developed exophthalmos. A diagnosis of Graves' disease, in addition to hypertension, was made and a subtotal thyroidectomy was performed. Her blood pressure at that time was 220/110. Following this there was no abatement in either the exophthalmos or the hypertension. Eleven years after the first operation, she was again operated on for recurrence of the symptoms of Graves' disease. At this time the blood pressure was still elevated 210/100.

She consulted me in December, 1928, complaining chiefly of shortness of breath, especially after exertion.

Her weight was 199 pounds (90.4 kg.). The obesity, however, was symmetrically distributed throughout the body. There were no indications of myxedema. The lungs were clear throughout. The heart was enlarged to the left. The rhythm was regular. A systolic blow was audible at the apex. The second aortic sound was accentuated and ringing in quality. The blood pressure was 225/120. The liver and spleen were not palpable and no masses were felt. The urine contained no sugar or albumin, or other abnormal constituents. The eyes were equally exophthalmic and showed no retinal pathology. The basal metabolic rate was plus 4 per cent.

She was seen again in April, 1929, at which time her weight was 200 pounds (90.9 kg.) despite dietary restriction, and the blood pressure 200/100. The basal metabolic rate was minus 2 per cent.

She was examined at irregular intervals thereafter, averaging about twice a year until April, 1933. During this period her condition showed no noteworthy change. The weight remained at about 200 pounds (90.9 kg.) and the blood pressure 200-220 systolic and 100-110 diastolic. The urine was consistently free of abnormal constituents. The specific gravity of occasional specimens was 1.024 and 1.020. The basal metabolic rate remained within normal limits. Orthodiascopic examinations indicated enlargement of the left ventricle.

In January, 1935, she complained of indefinite precordial pains, especially after exertion. The blood pressure was 190/100. Examination of the heart revealed no striking change. An electrocardiogram showed inversion of the T-wave in Lead I and left axis deviation. The urine was negative. The blood urea nitrogen was 20.4 mg. and sugar 98 mg. per 100 c.c. blood. The weight was 185 pounds (84 kg.).

In September she complained of epigastric distress, especially after eating. She had lost 10 pounds (4.5 kg.) in weight and had observed some increase in her dyspnea after exertion. Examination revealed a drop in blood pressure to 170/90. The soft blowing systolic murmur had become more pronounced and occasional extrasystoles were heard. The liver was not palpable, but for the first time the spleen was felt. A barium series done shortly after this showed a constant defect along the greater curvature in the midportion of the stomach. The enlarged spleen was clearly visualized. There was no obstruction or delayed emptying of the stomach or any part of the gastrointestinal tract. The possibility of carcinoma of the stomach with extension to the gastrosplenic omentum and invasion of the spleen was considered remote.

On March 26, 1936, the patient was suddenly seized with an attack of dizziness, following which she vomited fresh blood. The hematemesis recurred, and she had several tarry bowel movements. She was removed to the Hospital for Joint Diseases, where in spite of all efforts including two direct transfusions, she died of exsanguination four days later. An autopsy was performed one hour post mortem by Dr. C. J. Sutro.

Autopsy Findings.—Only the pertinent findings are herein recorded:

"Heart: The organ is in its normal anatomical site. The pericardial sac contains a small amount of clear fluid. The lining is everywhere smooth and glistening. The heart weighs 300 grams. Most of the enlargement is due to hypertrophy of the left heart. The right auricle and ventricle are somewhat larger than normal. Their endocardial linings are everywhere smooth and glistening. The tricuspid and pulmonary valves show nothing very striking. The cavity of the right auricle and ventricle is only a little larger than normal. The myocardium of the left ventricle measures in places from 2 to 3 cm. The endocardial lining of the left heart is everywhere smooth and glistening. The papillary muscles of the left ventricle are hypertrophied to almost twice the normal size. The chordae tendineae are shortened. The mitral and aortic valves present nothing very striking. There is a small amount of calcification at the aortic ring. The commissures are normal in appearance. The mouths of the coronary arteries are patent. The sections of these vessels show only scattered atheromatous plaques. There is no stenosis present.

"Microscopic section shows myocardial degeneration; slight coronary sclerosis without narrowing.

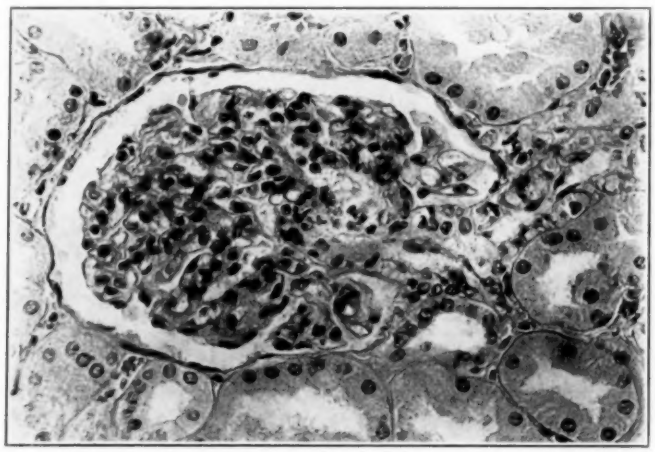

Fig. 1.—High power. Illustrating typical architecture of both kidneys. Normal glomeruli and arterioles.

"Lungs: A few thin fibrous adhesions are present in the right and left pleural cavities. Otherwise there are no abnormalities.

"Vascular System: The aorta is elastic throughout. Its arch is dilated to a very slight extent. An atheromatous plaque attached to one end of the intima is seen at the summit of the arch of the aorta. The thoracic and lumbar portions show a few atheromatous plaques with occasional calcification.

"Liver: The organ is in its normal anatomical site. It weighs 3,000 grams. Its free margin is two fingers below the ninth rib in the right mid-clavicular line. The liver is brown-yellow in color. The capsule of Glisson is uniformly wrinkled; beneath it can be seen closely set brown to yellow nodules. These measure from 2-5 mm. in diameter. The entire liver is composed of these colored small nodules. The caudate and quadrate lobes are also involved by a similar pathologic process. Here too, are present small yellowish nodules. The liver is firm to the touch. Cross-section shows the presence of the above described nodules. No large mounds of liver tissue are to be seen. The hepatic ducts in the liver show no closure at any point.

"Microscopic sections show normal architecture completely replaced by pseudolobules of varying size, separated by connective tissue which is infiltrated by many small round cells and an occasional polymorphonuclear leucocyte. The liver cells show degeneration with conspicuous vacuolization.

"Gastro-Intestinal Tract: The esophagus is in its normal anatomical site. Its mucosa is discolored reddish and numerous recent clots are seen on it. The stomach is of average size. The entire mucosa is red in color. Large clots are seen in the lumen of the stomach. The rugae are somewhat atrophic. Along the lesser curvature is seen an ulceration measuring 2½ cm. in diameter. The tissue about the ulceration is markedly thickened so that the wall of the stomach measures from ½ to ¾ of a cm. in thickness. No vessels are seen in or about the ulceration. No perforation is found. The duodenum shows nothing very striking. Large blood clots are seen in the jejunum and ileum. The mucosa of the small intestine shows nothing very striking except for reddish discoloration. The large intestines also contain clots.

"Microscopic section through the edge of the gastric lesion shows typical peptic ulcer.

"Pancreas: Shows nothing abnormal.

"Spleen: The organ is in its normal anatomical site. It weighs 460 grams. Part of the capsule is thickened. This presents itself as a 'cake icing' surface. Section shows indistinct Malpighian corpuscles. The organ is firm to the touch. It has a deep brown color. There is an increase in stroma. The pulp is firm. The vessels show nothing striking.

"Microscopic sections show perisplenitis with hyalinization and considerable iron pigment deposition, sinus hyperplasia with some fibrosis of the pulp.

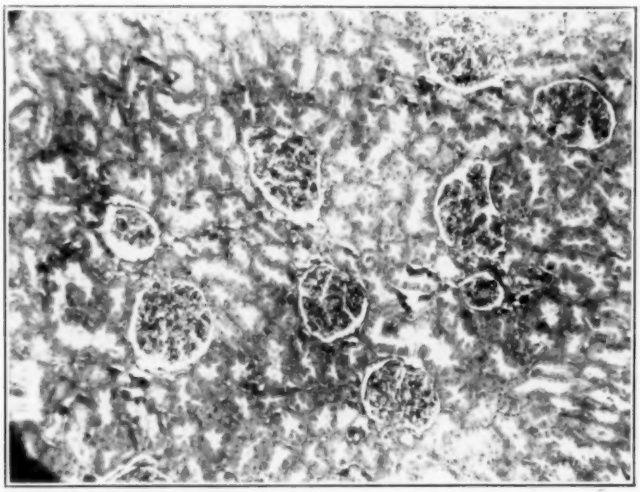

Fig. 2.—Low power. Same as Fig. 1.

"Adrenals: Both adrenals are in their normal anatomical sites. They are of average size and shape. Sections show that their cortices contain a large amount of yellow material.

"Kidneys: Both kidneys are of average size and shape. They are normally situated. The perirenal fat is normal in quantity and has an orange color. The capsules strip with ease. Section shows that the cortices are normal in width. The cortico-medullary junctions are sharp. The calyces are normal in size. The pelves show nothing very striking. The ureters are average width. The mucosae are everywhere smooth and glistening.

"Microscopic section shows no significant change." (Figs. 1 and 2.)

SUMMARY

The patient had hypertension for more than a score of years; she succumbed at the age of 50 to an apparently independent condition. At autopsy the heart weighed 300 gm. and the kidneys on gross and microscopic section were free of any abnormality characteristic of hypertension. In addition the patient had had Graves' disease necessitating partial thyroidectomy at the age of 30 and again at 41, which was evidently a superimposed condition and not a cause of

the hypertension, because the blood pressure was known to be elevated several years before symptoms of hyperthyroidism appeared and continued markedly elevated for many years after thyroidectomy, during which interval the metabolic rate was consistently normal.

A gastric ulcer had been present and healed spontaneously with very few symptoms during life to indicate its existence. The patient also had cirrhosis of the liver. This was probably a sequela to the hepatic degeneration which has been found to occur in Graves' disease and the splenomegaly in turn was secondary to this.[2]

DISCUSSION

It appears that cases, such as the one herein described, are rare. A few have been referred to in the literature.[3] However, the fact that they do occur is of the greatest importance. They establish the independence in at least certain cases of hypertension and renal impairment.

There is no proof here that the arteriolar sclerosis which constantly accompanies essential hypertension[4] is the result of the high blood pressure. To argue whether this patient would eventually have developed arteriolar thickening would be speculation. The findings, however, in the case herein reported indicate that essential hypertension may arise and continue independently of any renal impairment.

CONCLUSIONS

A case of essential hypertension of more than twenty-five years' duration complicated by recurrent Graves' disease is reported.

Evidences of renal impairment were consistently absent.

At autopsy the kidneys showed no sclerosis of the arterioles or other pathology.

This case establishes the possibility that normal arterioles including those of the kidneys are not incompatible with hypertension of many years' duration.

This case indicates further that essential hypertension may arise and continue from causes still unknown but independent of any functional or organic renal pathology.

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THE EFFECT OF LIVER EXTRACT UPON POLYCYTHEMIA VERA*

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THE demonstration by Minot and Murphy[4] of a liver fraction, effective in the treatment of pernicious anemia, has focused attention upon this disease as a deficiency disease. The proof that this anemia is apparently due to the deficiency of a certain factor quite logically led to the theory that polycythemia vera is a disease in which this factor is present in excess. This theory was supported by Morris, Schiff, and Foulger[5] and by Briggs and Oerting,[2] who described improvement in the blood count in patients of polycythemia following gastric lavage. This has also been supported by Patton, Allardyce, and McKeown,[7] who describe polycythemia vera as the antithesis of pernicious anemia. More recently Baráth and Fülöp[1] have described an increase in the amount of the hemopoietic factor in the gastric juice of patients with polycythemia vera as compared with normal individuals.

A number of years ago it occurred to us that if polycythemia vera were due to an excess of a hemopoietic, antianemic factor, then treatment of such patients with liver extract would further increase the blood count of patients with polycythemia vera. Two observations were made in 1931 and a third observation was made in 1936. The blood counts were carried out in the usual manner, and the hemoglobin estimations were carried out with the Haden-Hausser hemoglobinometer. A brief summary of these patients is as follows:

CASE 1.—G. P., No. 33448, male, aged 65 years, was admitted to the hospital November 21, 1930, and was dismissed February 13, 1931. This patient on admission showed a red cell count of 8,930,000, white cell count of 11,550, and hemoglobin of 110 per cent. Five days after admission the patient was placed on Lilly's liver extract, No. 343, two capsules three times a day. This therapy was continued until the patient's dismissal from the hospital at which time his red cell count was 6,660,000.

	R.B.C.	W.B.C.	HEMOGLOBIN	RETICULOCYTES (PER CENT)
11/21/30		11,550	110	
11/22/30	8,930,000			
11/23/30	8,620,000	13,650	112	
11/30/30				0.8
12/ 1/30	7,360,000	12,050	109	11
12/ 2/30	6,790,000			None
12/ 4/30	7,030,000			0.14
12/ 5/30	6,660,000			0.26
12/ 6/30	7,050,000			
12/ 8/30	7,420,000	11,400	105	

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CASE 1.—CONT'D

	R.B.C.	W.B.C.	HEMOGLOBIN	RETICULOCYTES (PER CENT)
12/ 9/30	7,630,000			
12/10/30	7,720,000			None
12/11/30	7,500,000			None
12/12/30	7,100,000			None
12/13/30	6,540,000			None
12/15/30	7,100,000			None
12/16/30	7,200,000			
12/17/30	6,840,000	11,800	111	None
12/18/30	8,280,000			
12/19/30				None
12/27/30	7,560,000			
12/30/30	7,540,000			
1/ 2/31	6,000,000			
1/ 6/31	6,370,000			
1/ 7/31	6,440,000			None
1/ 8/31	6,620,000			None
1/ 9/31	6,300,000			None
1/12/31	5,760,000	10,100	104	
1/13/31	5,400,000			None
1/14/31	5,740,000			
1/15/31	5,650,000			
1/16/31	5,600,000			
1/19/31	5,740,000			None
1/20/31	5,570,000			None
1/21/31	6,300,000			None
1/22/31	5,840,000			None
1/31/31	6,720,000	11,000	106	
2/ 6/31	6,430,000			
2/ 7/31	6,540,000			
2/11/31	6,660,000	10,200	105	

CASE 2.—L. F., No. 33926, female, aged 56 years, was admitted to the hospital January 12, 1931, and was dismissed January 14, 1931. This patient was admitted to the hospital following a severe epistaxis at which time the cell count was red blood cells 6,350,000, white blood cells 21,200, and hemoglobin 97 per cent. This patient was placed on liver extract, No. 343, two capsules three times a day, on January 26, 1931, and the treatment continued until January 9, 1933. The blood picture showed the following findings:

	R.B.C.	W.B.C.	HEMOGLOBIN	
1/21/31	6,170,000	18,000	87	
1/28/31	6,910,000	22,500	88	
2/ 1/31	7,900,000	19,400	103	
2/11/31	6,710,000	15,450	90	
4/15/31	7,350,000	18,800	97	
5/ 6/31	8,090,000	18,650	100	
6/ 3/31	8,200,000	18,200	103	
7/21/31	8,410,000	17,850	97	
8/18/31	7,400,000	1,900	94	
9/23/31	8,120,000	18,850	100	
10/21/31	8,630,000	19,450	100	
11/15/31	6,900,000	12,400	100	(15.5 gm.)
11/25/31	8,950,000	17,200	103	
12/ 2/31	8,760,000	17,900	104	
12/ 9/31	8,380,000	18,350	103	
12/23/31	9,020,000	14,350	103	
1/ 6/32	8,450,000	21,300	97	
2/ 3/32	7,700,000	8,900	90	
3/23/32	7,940,000	12,250	94	(14.5 gm.)
4/15/32	9,090,000	12,000	97	
1/ 9/33	7,640,000	16,100	103	

CASE 3.—J. L., No. 69390, male, aged 55 years, was admitted to the hospital November 9, 1937, and was dismissed November 30, 1937. This patient was admitted to the hospital showing a blood picture of R.B.C. 8,960,000, W.B.C. 10,200, and hemoglobin 148 per cent (23 gm.). This patient was given a highly potent liver extract in the form of Reticulogen, 0.5 c.c.; and these injections were given every other day. The blood counts were as follows:

	R.B.C.	W.B.C.	HEMOGLOBIN	RETICULOCYTES (PER CENT)
11/ 8/37	8,960,000	10,200	148	
11/11/37	9,200,000	7,500	154	2.5
11/12/37	8,840,000	12,350	154	2
11/13/37	9,000,000	12,000	154	2.2
11/15/37	8,700,000	13,200	153	1
11/16/37	9,010,000	13,700	158	1.85
11/17/37	8,990,000	14,500	160	1.54
11/18/37	9,100,000	10,400	145	0.66
11/19/37	8,890,000	12,000	156	1.2
11/20/37	8,340,000	12,200	154	1
11/22/37	4,820,000	12,400	150	0.6
11/23/37	8,200,000	12,800	154	0.3
11/24/37	8,500,000	12,100	150	0.6
11/26/37	8,900,000	12,200	154	1.3
11/27/37	9,020,000	12,700	148	0.8
11/29/37	8,600,000	11,900	146	0.4
11/30/37	7,950,000	13,750	152	1

The above findings were interesting to us in view of the previous publications of Stephan,[8] Ogawa,[6] and Hitzenberger.[3] These authors described a very satisfactory fall in the red blood cell count of patients with polycythemia vera following treatment with liver extract. Indeed, this treatment is recommended by them for polycythemia vera, their views being, at least theoretically, the antithesis of those previously cited. Our observations have led us to conclude that the liver extract is without any specific effect on the red blood cell count, the hemoglobin or the reticulocyte count in patients suffering from polycythemia vera. Such variations as we saw, we felt were only the variations untreated patients often show and bore no relationship to treatment with liver extract.

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THE REDUCTION OF PRONTOSIL SOLUBLE BY URINE*

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IT WAS observed that, upon standing, urine specimens from patients being treated with prontosil frequently decolorized the dye they contained. Investigation revealed that upon standing for more than one day normal urine specimens, collected at random under nonsterile conditions, were capable of decolorizing appreciable amounts of dyestuff. Addition of phenol or mineral acid stopped the reduction process, as did boiling the urine for five minutes prior to the addition of the dye. Two urine specimens taken by catheter did not decolorize sterile dye solutions. It has been suggested[1] that the in vivo activation of prontosil may result from bacterial reduction processes. In the present instance, bacterial intervention functions mainly in increasing the urinary pH by alkaline fermentation. Cultures of *B. coli* did not decolorize very dilute sterile dye solutions. Addition of ammonium hydroxide, sodium hydroxide, or sodium carbonate accelerated the decolorization process.

When added to normal urine in concentrations of 15 to 25 mg. per cent, prontosil was consistently reduced if 25 c.c. of the urine was boiled for fifteen to thirty minutes in the presence of sufficient alkali to darken the hue of the prontosil. A wide variety of urine samples was studied. In general, normal urine was not capable of decolorizing concentrations of the dye above 25 to 30 mg. per cent under these conditions. This value varied somewhat with different samples, but more markedly with the conditions under which the reduction was carried out. Under these conditions uric acid, creatinine, cevitamic acid, and cysteine did not reduce the dye. However, glucose which normally occurs in urine in concentrations up to 0.4 per cent is known to reduce azo dyes in alkaline media. In agreement with the assumption that glucose is the reducing substance active in normal urine, it was observed that urine from diabetic patients decolorized higher concentrations of the dye, and similarly, adding glucose to the urine produced reduction of increased amounts of the dyestuff. Other reducing sugars, fructose, lactose, maltose, etc., produced similar reductions.

Air oxidation impedes the reduction process. Standard solutions of glucose (1.0M and 0.1M) were titrated from a pipette into a casserole containing 10 c.c. of boiling standard prontosil solution and 1 c.c. of sodium hydroxide (1 per cent). For high concentrations of the dye, the glucose was added in one or two molar amounts, and the solution was boiled for three minutes after each addition. For lower concentrations ten moles of glucose were added at a time. Dye concentrations of 0.0001M and 0.0005M required 200

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to 250 moles of glucose, 0.001M required 70 to 90 moles, and 0.01M dye required only 17 to 20 moles of glucose for complete decolorization. A similar influence of air oxidation in the urinary reduction of the dye at room temperatures was evident: surface layers of the urine remained red much longer than the lower layers.

Attempts to standardize the use of prontosil for the determination of urinary sugar were discontinued since unsatisfactory end points were obtained. The final color depends upon the concentrations of both reactants and the pH. Alkaline solutions of glucose become colored from a yellow to a deep red brown, and this color varies directly with the glucose concentration and is darkest in alkaline media. The resultant aminonaphthol is readily air oxidized in alkaline medium to a quinonoid green color, which varies in intensity directly with the dye concentration, the time of heating, the extent of aeration, and the pH. A similar color change was observed in the reductions of the dye by normal urine which was allowed to remain at room temperatures. After complete reduction of the dye, aeration produced a greenish cast in the specimens, indicating the absence of hydrazines.

It has been shown that prontosil[2] and similar dyes[3] are reduced in the animal. Reduction may also occur in urine. Complete recovery of the injected dye in excretion studies necessitates that these reduction processes be considered.

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LABORATORY METHODS

CULTURE VERSUS GUINEA PIG INOCULATION IN THE DIAGNOSIS OF TUBERCULOSIS*

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IT WAS not until Dorset's description of his egg medium in 1902 that attention was focused upon the potentialities of cultural methods for the successful isolation of tubercle bacilli from contaminated material. It is beyond the scope of this paper to discuss all the media and their various modifications that have been brought forth since that time. Suffice it to say that the techniques of Petroff,[1] Corper,[2] Sweany and Evanoff,[3] Lowenstein,[4] and Petragnani,[5] among others have proved satisfactory in the hands of some investigators and unsatisfactory in others.

Herman, Hansman and De Capito,[6] using Corper's medium, studied a large series of 537 cases with both guinea pig inoculations and cultural methods. Of these, 48 proved positive by both methods. In an additional 28 cases the guinea pig alone became positive, whereas in 7 instances the culture alone became positive. Naturally, they concluded that animal inoculation was a more reliable procedure. Nevertheless, upon analyzing their cases, one notes that guinea pig fatalities accounted for twice as many failures as culture tube contaminations, drying of media, and breakage. The authors did not state whether they divided the material equally between the animal and the culture tubes.

In 1931 Stadnichenko and Sweany[7] compared the results of both methods in a series of 200 specimens of sputum that were negative on stained preparations of concentrated material. There were 36 instances in which a positive result was obtained by either one method or the other. The guinea pig method was responsible for 33 positive results and the culture method for only 3. The medium used was that of Sweany and Evanoff. There were no statistics on guinea pig mortality or contamination of cultures. It is not clear how many tubes were inoculated or the relative amounts of material apportioned to culture tubes and guinea pigs.

Feldman[8] found in the examination of clinical material that inoculation of animals is preferable because of its greater certainty. It is difficult to follow his line of reasoning because conclusions were based on a small series of 46 cases, 41 of which were positive by direct smear and only 5 negative. Of these 5, 2 were positive by animal alone and 3 were positive by both animal and

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culture. In the remaining 41 cases, as expected, comparable results were obtained by both methods. Three different media totaling 18 tubes were used in each test.

Later the same author, in collaboration with Magath,[9] reported on the examination of 100 specimens negative by direct smear. The methods of Corper, Sweany, and Miraglia were tested. Two-thirds of the sediment was injected into guinea pigs and the remainder was seeded onto at least 8 tubes of media. Contaminations ran from 5 per cent to 90 per cent, depending on the digestant and the media used. There were 14 cases in which the animal alone was positive and only 4 cases where the culture yielded tubercle bacilli. Failure to recover the tubercle bacilli in ten instances may have been due to the contaminated cultures.

On the other hand, Norton, Thomas, and Broom,[10] using the Petragnani medium, concluded that the culture was the more sensitive method. From 151 cases negative by smear, 54 positives were obtained, 35 by both guinea pig and culture, 16 by culture alone, and 3 by guinea pig alone. Hirschberger[11] found cultures more sensitive in a series of 69 cases. Corper's, Hahn's and Miraglia's media were selected for this study and proved very satisfactory.

From this brief review of pertinent literature one is left with the impression of a wide diversity of opinion concerning the efficacy of cultural methods. It is also apparent that unless every step is scrupulously supervised, the end results of such an investigation are apt to be fruitless or at least indecisive. For that reason, it would not be unpardonable to criticize the soundness of some of the methods of study and the technique employed therein.

There has been a fairly general tendency to inoculate the guinea pig with greater quantities of the suspected material than the culture tubes. Frequently two to three different media involving 8 to 16 culture tubes were tested simultaneously, thus allowing a mere fraction of the total amount for each tube. What is more important, where the cultures alone proved positive, few individuals determined the pathogenicity of these acid-fast organisms by animal test.

THE PRESENT STUDY

It was the purpose of the authors to select one suitable medium and submit it to a prolonged, rigidly controlled, parallel study with guinea pig inoculations. In previous culture work in this laboratory, the Petragnani[5] medium had proved very satisfactory and therefore was our logical choice.

Specimens were collected from patients in the Out-Patient Department and medical pavilions of the New York Hospital. All were from proved or suspected cases of pulmonary tuberculosis. A total of 200 specimens comprising 137 sputa, 38 gastric washings, 21 pleural fluids, and a miscellaneous group of 4 was studied according to the procedure outlined below. Smears made from the concentrated material of 171 specimens revealed no acid-fast organisms. Fifteen were slightly positive on smear and 14 markedly so. These latter 14 were used at the beginning of this study to test out the method on known positives and as a further check on the efficacy of the medium chosen.

TECHNIQUE

Collection and Preparation of Specimens.—Sputum was collected in one container for three consecutive days. Gastric contents were taken on a fasting stomach, using as little saline as possible. Pleural fluids were placed in sterile flasks containing sodium citrate; 1 c.c. of a 10 per cent solution to every 10 c.c. of fluid. Purulent pleural fluids were spun down in the centrifuge and the serum discarded; thus the sediment was left with which to work. The specimens were then transferred to sterile bottles and treated as follows:

1. Addition of an equal quantity of 4 per cent sodium hydroxide.
2. Agitation in a shaking machine for one-half hour.
3. Incubation for one-half hour at 37.5° C.
4. Centrifugalization in sterile tubes for one-half hour at 2,500 revolutions per minute.
5. Decantation of supernatant fluid.
6. Slide made from sediment.
7. Neutralization of sediment with a few drops of 8 per cent hydrochloric acid.
8. Resuspension of sediment in sterile saline up to 3 c.c. and introduction of 2 or 3 sterile beads.
9. Agitation once more in shaking machine for ten minutes.
10. Equal division of material for animal inoculation and cultures.

Preparation and Examination of the Slide.—A small loopful of the sediment was removed sterilely and distributed as evenly as possible over one-half the surface of the slide. When the sediment was very scant, as in gastric content digests, the slide was first prepared with a thin layer of egg albumen as a fixative. The slides were stained by the Ziehl-Neelsen method and counterstained with a 1 per cent solution of brilliant green in 1:10,000 potassium hydroxide. All smears were examined at least ten minutes and checked by another worker.

Culture and Guinea Pig Inoculations.—One-half (1.5 c.c.) of the resuspended, finely broken up sediment was seeded equally on 3 slants. The tubes, which were sealed with cotton plugs dipped in paraffin, were placed in the incubator for two or three days in an almost horizontal position. This insured even distribution of the material over the surface of the medium and gave the tubercle bacillus an opportunity to become adherent to the slant. The tubes were then sealed with cellophane wrappers as a further aid in preventing the medium from becoming dehydrated. The remaining 1.5 c.c. of material was injected subcutaneously in the inguinal region of the previously tuberculin tested guinea pig. Each guinea pig was placed in an individual cage.

Examination of the Culture.—All culture tubes were examined macroscopically once a week. At the end of the second week microscopic examination was started. One tube of each set of three was opened and the surface scraped with a platinum loop. This was washed off in a small amount of saline on a slide prepared with egg albumen. (New slides were used exclusively.) The tube was sealed and replaced in the incubator. The second tube of each set was examined in the same way at the end of the third week, and the third tube at the

end of the fourth week. If macroscopic growth had not appeared by this time, the three tubes were examined in the same order at the end of the fifth, sixth, and seventh weeks. The cultures were discarded at the end of the eighth week. The date of the first appearance of growth, both microscopic and macroscopic, was recorded; likewise the presence and nature of contaminants.

Examination of the Guinea Pig.—At the end of the second week after inoculation a tuberculin test was done on each guinea pig, using 0.1 c.c. of a 5 per cent dilution of old tuberculin intradermally. This was repeated weekly until a two-plus Mantoux appeared, or continued through the eighth week if the test remained negative. In any case the animal was sacrificed two days after the final test. An autopsy was performed on each pig and the presence of tubercle bacilli noted in the tissues before a positive interpretation was accepted as final.

RESULTS

As will be noted in Table I, of the 171 specimens negative on stained preparations, 136 failed to become positive by culture or to produce tuberculosis in the guinea pig; 22 became positive by both culture and guinea pig inoculation; 9 by culture only, and 4 by animal test only. In most cases growth was

TABLE I

COMPARISON OF RESULTS OF CULTURES WITH GUINEA PIG INOCULATIONS ON CONCENTRATED SPECIMENS NEGATIVE BY SMEAR

NO. OF SPECIMENS	NEG. BY BOTH GUINEA PIG AND CULTURE	POS. BY BOTH GUINEA PIG AND CULTURE	POS. BY CULTURE ONLY	POS. BY GUINEA PIG ONLY
119 Sputa	95	14	7	3
17 Pleural fluids	11	5	0	1
35 Gastric contents	30	3	2	0
Total 171	136	22	9	4

noted on all three tubes of each set. In each of the nine instances when the culture alone yielded acid-fast bacilli, a portion of the visible growth was inoculated into normal guinea pigs. All of these animals became infected, gave positive Mantoux tests, and showed extensive tuberculous lesions at autopsy.

An additional 15 specimens, slightly positive on stained preparations, were also tested. Thirteen gave positive results by culture and animal inoculation. Two failed to become positive by either method.

Where the specimens were negative by smear, an average of thirty days was required for the culture to produce macroscopic growth, and an average of thirty-three days for the guinea pigs to show positive Mantoux tests. Where the specimens were slightly positive by smear, the time required for the cultures and animals to become positive was approximately one-half as long as when the smears were negative. As a rule microscopic growth was discovered about one week before characteristic colonies were visible.

As will be seen in Table II the percentage of guinea pig fatalities was considerably greater than the percentage of complete culture set contaminations. However, the actual number of individual culture tubes contaminated was 25 out of a total of 558 tubes, or 4.5 per cent.

TABLE II

AN ANALYSIS OF UNCOMPLETED* TESTS DUE TO GUINEA PIG FATALITIES AND CULTURE CONTAMINATIONS

NO. OF SPECIMENS TESTED	UNCOMPLETED TESTS*		PERCENTAGE UNCOMPLETED TESTS	
	GUINEA PIGS	CULTURE SETS	GUINEA PIGS	CULTURE SETS
186	11	2	6	1

*Uncompleted tests refer to procedures which had to be abandoned because of the death of the animals or the contamination of all three tubes of a culture set before the time limit of eight weeks.

The contaminants encountered were of two kinds: those forming discreet colonies, and those forming spreading colonies. The latter gave the most difficulty because once they appeared, the entire surface of the slant was quickly overrun. The former, however, in only a few instances, rendered the culture tube worthless for the identification of colonies of acid-fast bacilli.

COMMENT

In the early part of this article we have referred to the uncertainties that exist in the minds of investigators concerning the relative value of cultural methods and guinea pig inoculations. We have also placed great stress on the necessity of carefully controlling each step in any procedure used to determine the superiority of one method over the other.

Altogether there were 171 specimens negative on examination of the stained preparation. Of these, 31 became positive by culture as compared with 26 by guinea pig inoculation. Such an outcome indicates that the culture medium possesses a slightly greater sensitivity.

We have little comment to offer concerning the two occasions in which the cultures did not yield growth when the slides were positive. The corresponding guinea pigs succumbed to intercurrent infection in too short a period for development of tuberculous lesions. Although the culture method must be considered a failure in these two cases, it should be remembered that occasionally guinea pigs fail to contract tuberculosis after injection with faintly positive specimens.

The low incidence of contaminations in this series is noteworthy (4.5 per cent of the total number of tubes). We are convinced that this outcome could be attributed in a large degree to the fine state of dispersion of the material introduced upon the slants. Intimate contact of all the particles with the bacteriostatic agent was thereby assured.

Culture methods have certain very obvious economic advantages. In many communities, laboratories find it impossible to obtain guinea pigs. Where such a situation exists, cultures are recommended as a valuable supplement to routine slide examination.

In laboratories where guinea pigs are readily available and in routine use the culture technique affords additional diagnostic aid.

CONCLUSION

Petragnani's medium is an effective agent in the isolation of tubercle bacilli from contaminated material. In our hands, the cultural method employing this medium appears to possess a slight superiority over the guinea pig inoculation.

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MODIFIED VAN ALLEN HEMATOCRIT TUBE PROVIDING FOR AUTOMATIC VOLUME ADJUSTMENT OF THE BLOOD SAMPLE*

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THE pipette here described was designed for the determination of the volume of cells in blood according to the Van Allen (1925) method, and to facilitate measurements of cell volume in a method for the determination of red blood cell fragility which will be reported later. It offers advantages over the original Van Allen hematocrit tube by providing quicker and more precise measurement of the blood sample: this being accomplished by automatic volume adjustment of the sample in a capillary tube similar to that used in the Trenner diluting pipettes employed for counting blood cells.

The Van Allen hematocrit method possesses the advantage of requiring only a small amount of blood, obtainable by skin puncture; furthermore, in this method, packing of the cells to constant volume can be attained in a relatively short time and at a relatively low centrifuge speed because the blood is diluted in a salt solution having lower specific gravity and viscosity than the blood plasma. Against these advantages may be cited the objections that the rubber sealing device may permit leakage during centrifugation, and that the diluting fluid, although apparently isotonic for normal blood, may not be isotonic for bloods from subjects with various pathologic conditions. Such considerations as these must be weighed carefully when a choice is made of any one of the many hematocrit methods which have been devised for cell volume measurements.

*From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati.

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In the procedure as originally described by its author, blood is drawn into the Van Allen hematocrit tube up to the 100 mark, excess blood is wiped from the tip, and a diluting fluid (1.3 per cent sodium oxalate solution) is drawn into the tube until the bulb is about half filled. The tube is sealed—either with a rubber band or with a spring clip device which holds a rubber cushion against

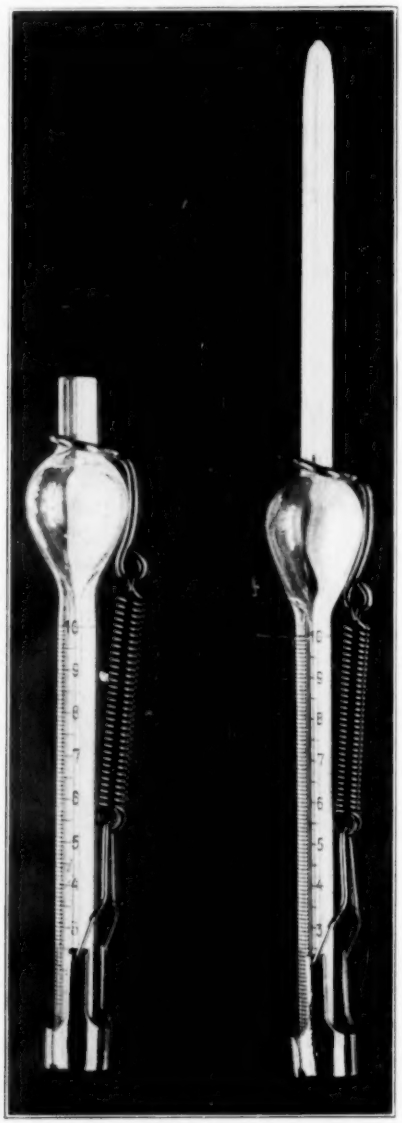

Fig. 1.

Fig. 2.

Fig. 1.—Original and modified Van Allen hematocrit tubes.
Fig. 2.—Diagram showing construction of the modified tube.

the tip—and is then centrifuged. The height of the column of packed cells, read from the graduations on the stem, gives the percentage volume of cells in the sample.

In practice, accurate adjustment of the blood sample in the original tube is somewhat tedious and subject to error, inasmuch as the broad flat tip makes

difficult the wiping of excess blood from the end of the tube without pulling the column of blood away from the 100 mark. The modified tube shown in Fig. 1 was designed primarily to overcome this difficulty. For such a modification it was, of course, not possible merely to substitute the Trenner type of capillary tube for the graduated stem of the original tube, because the blood cells when centrifuged would then settle at the flat shoulder inside the bulb instead of in the graduated stem where the column of cells is measured. Both ends of the pipette, therefore, were utilized; one for measurement of the blood sample, the other for measurement of the column of packed cells after centrifugation.

Construction.—The capillary tube A is tapered at the tip, to facilitate wiping away of excess blood, and at the opposite end it terminates in a ground and polished surface at right angles to the longitudinal axis. The volume of the bulb has been made (arbitrarily) approximately 100 times that of tube A. It is formed separately, blown from tube B in order to provide a smooth funnel-shaped opening into the latter, and is fused to the shoulders of tube A. The graduated portion of tube B has a volume exactly equal to that of tube A, and is marked with 100 divisions numbered by tens from the distal end.

In use, a rubber suction tube with mouthpiece is first fitted to the end of tube B. With the pipette held horizontally or with the B end slanted downward, blood is drawn into tube A by gentle suction until it will flow by capillary to fill the tube A completely. After excess blood is wiped from the tip, diluting fluid is drawn into the bulb by suction until the bulb is nearly filled. An air bubble left in the bulb aids the mixing of the blood with the diluting fluid. The suction tube is then removed, the stem B is sealed with a spring clip (the same as that used with the original Van Allen tube) which holds a rubber cushion against the blunt end, and the tube is centrifuged with the sealed end downward. The cells are thus packed into the graduated stem B, and there measured directly in percentage of the volume of the sample by reading of the graduations.

Since a rather thin edge of the bulb is fused to the outside of the shoulder of tube A, this modified tube is necessarily more fragile than the original form, and there is greater danger of breakage at high centrifuge speeds. In order to avoid torsion stress, the tube should be placed in a centrifuge cup which holds it in an exactly perpendicular position. The International Centrifuge, type SB, No. 1 or 2, may be used with the 15 ml. centrifuge tube carriers. The rubber cushions usually placed at the bottom of the metal cups are not needed. These cups can be filled with water above the shoulder of the bulb, to give additional support in counterbalancing the centrifugal force against the bulb.

SUMMARY

The Van Allen hematocrit tube has been modified to provide quicker and more precise measurement of the blood sample, by utilizing the principle of automatic volume adjustment of the sample in a capillary tube similar to that used in Trenner hemacytometer pipettes.

This pipette and spring clip sealing device is obtainable from the Arthur H. Thomas Co., Philadelphia.

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AN IMPROVED MANTEL FOR BACTERIA-PROOF FILTER CANDLES*

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THE glass mantels commonly used with bacteria-proof filter candles have several inherent disadvantages: they are breakable, the liquid undergoing filtration is exposed continuously to air-borne contaminants, and repeated refillings requiring frequent attention are necessary when large volumes are filtered. A metal mantel free from these disadvantages, and particularly serviceable for the filtration of large volumes of products is shown in Figs. 1 and 2.

The mantel of which the dimensions are given in Fig. 1 will accommodate a 5 inch filter candle and a 4 liter filter flask. The metal used has been duralumin, which is obtainable in bars and tubing of the requisite sizes. The hole through the base of the mantel is threaded to fit the nipple of the candle. If a suitable tap is not available, one of the metal nuts always supplied with candles may be sweated into a hole countersunk in the "boss" of the base. The glass tube and rubber stopper shown may be replaced by a metal cap threaded to fit the top of the cylinder and provided with a suitable nipple and washer. The top of the flask upon which the mantel rests must be ground flat if necessary.

Three different types of rubber washers are used. The washer between the candle and the boss is cut from 1/4 inch sheet rubber packing provided with cloth insertion. It is used once and then discarded. The washer between the cylinder and the "boss" is a standard 2 inch hose washer. The large washer between the "boss" and the flask is cut from 5/16 inch grade A rubber sheeting. The latter two washers are used as long as they remain serviceable.

The set-up of the apparatus is shown in Fig. 2. Prior to sterilization, the mantel, candle, and suction flask are assembled, and the space between the inner wall of the base of the mantel and the outer wall of the flask is packed with cotton. The assembled apparatus with supply tube attached or not, as preferred, is then sterilized.

In operation one end of the supply tube with the clamp closed is attached to the inlet tube of the mantel, and the other end is immersed in the liquid to be filtered, which should be contained in a reservoir plugged with cotton. The system is thus protected from air-borne contaminants. The suction flask is connected to the vacuum line, and suction is applied with simultaneous downward pressure upon the mantel. When the collapse of the supply tube indicates exhaustion, the clamp is opened, the mantel fills, and the filtration proceeds automatically. With suitable precautions for the prevention of contamination,

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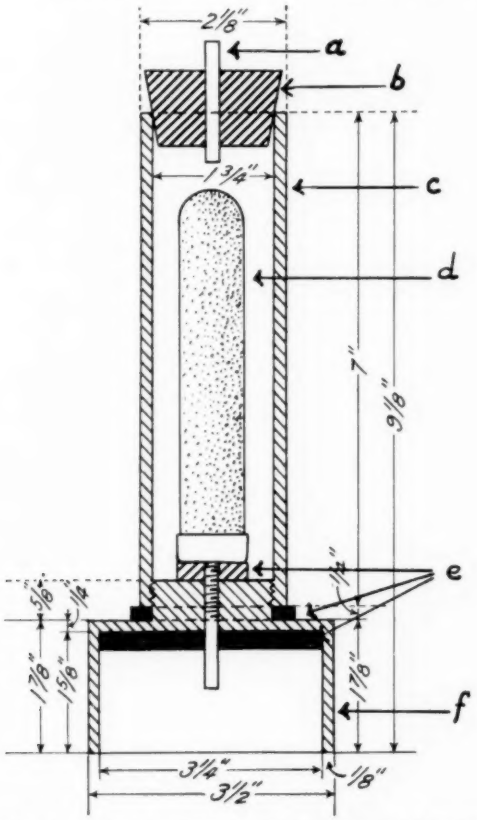

Fig. 1.—*a*, Glass inlet tube; *b*, rubber stopper; *c*, metal cylinder; *d*, candle; *e*, rubber washers; *f*, metal base.

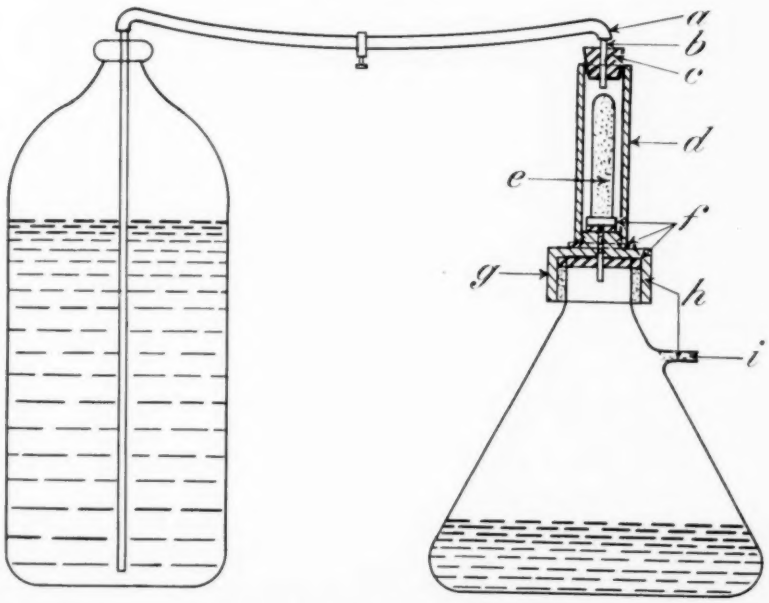

Fig. 2.—*a*, Rubber supply tube; *b*, glass inlet tube; *c*, rubber stopper; *d*, metal cylinder; *e*, candle; *f*, rubber washers; *g*, metal base; *h*, cotton; *i*, to vacuum line.

the mantel may be transferred from one flask to another. Since the only attention required is the turning on and off of the suction, a number of filters may be connected to a single reservoir and operated simultaneously.

The apparatus has been used chiefly for the filtration of intradermic tuberculin which contains 0.5 per cent phenol. During the past several years more than five million cubic centimeters have been filtered and the filtrates examined by cultural and microscopic methods. No contamination has been traced to a fault or defect in the mantel.

A NEW STOMACH TUBE FOR ORAL ADMINISTRATION IN RATS AND MICE*

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IN A SERIES of experiments on mercury intoxication, it became necessary to give rats and mice oral administrations of small, accurately measured doses of liquids. The customary method, using a small rubber catheter, was found to be neither accurate nor rapid enough for this work.

An instrument satisfactory for this study was made by modifying an 18 gauge hypodermic needle of length sufficient to pass into the stomach of the animal being used. The beveled point of the needle was removed on an emery wheel and the last 2 cm. of the end of the needle was covered with a layer of solder several millimeters thick. This end was then shaped on a lathe until a smooth blunt tip, about 2 mm. in diameter, with a gradual taper to the shank of the needle remained. For adult rats, a needle 4 inches in length allows insertion into the stomach. For mice a 20 gauge needle, 2 inches long with a ball tip of about 1 mm. thickness, should be used. The needle is attached to a syringe of capacity sufficient to hold the dose being administered.

TECHNIQUE

At first it is best to have two operators, although after a little practice one person can perform the operation. One operator holds the head and extends the tongue of the animal while the second inserts the needle into the stomach, manages the syringe, and holds the animal's tail.

The first operator takes a full grasp of the skin on the back of the animal, extending from immediately behind the ears as far down the back as a grip between the thumb and the full medial side of the index finger of the left hand allows. The middle or third finger of the left hand pins the left front leg against the animal's body. The thumb does not go around the throat but the full weight of the animal is maintained by the scruff of the neck. With the right hand, the tongue is grasped firmly with blunt dressing forceps and drawn out to the left side of the animal. This opens the mouth and allows the insertion of the needle deep into the pharynx. The tongue is released after the needle has passed over

*From the Research Laboratories of the George A. Breon & Co., Inc.
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the papilla at its base and the right hand may then be used to grasp the left cheek of the animal to draw the head and neck in line with the body if the animal insists on arching its neck.

The second operator holds the body of the animal straight by maintaining a steady pull on the animal's tail with the left hand. With the right hand he inserts the needle and operates the syringe. The index finger of the right hand should be used to bind the plunger of the syringe against the syringe wall to prevent leakage of the solution through the needle while inserting. The needle should be passed into the mouth in line with the animal's body and just to the left of the incisors with the blunt end against the palate. By a gentle lever action on the syringe forcing the head back as the end of the needle approaches the larynx, the needle passes easily over the glottis into the esophagus and enters the stomach without difficulty.

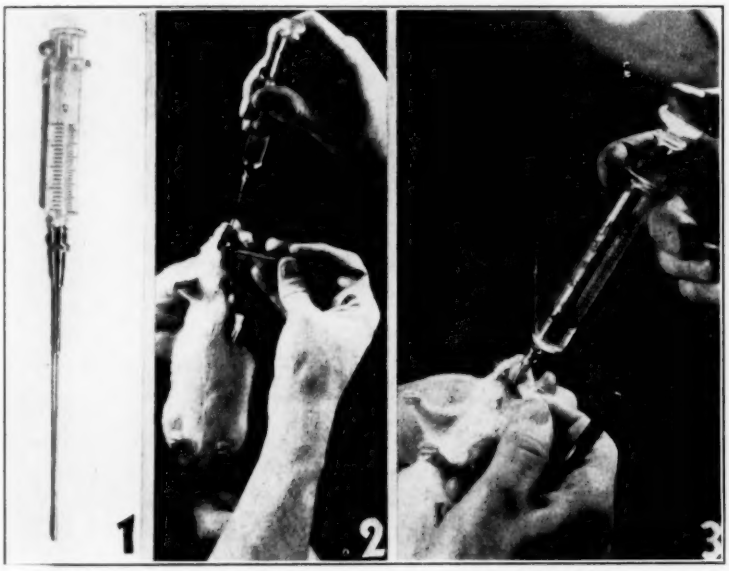

Fig. 1. Fig. 2. Fig. 3.

Fig. 1.—Stomach tube hypodermic needle with enlarged blunt tip.
Fig. 2.—Introducing tube into mouth of rat. Note extension of tongue.
Fig. 3.—Passing tube into stomach. Note straightening of neck by pull on cheek.

When the injection is made by one person, the left hand holds the animal by the scruff of the neck and the right hand inserts the needle by feeling the way over the tongue and using a gentle leverage with the syringe to force back the animal's head.

Two injections per minute can be accomplished easily with doses under 5 c.c. With larger doses time must be allowed for the stomach to relax, otherwise the esophagus will be filled and part of the injection lost through the mouth and nose. Since the perfection of this apparatus over 7,000 injections (as high as 300 injections on one rat) have been made in this laboratory without the loss of a single animal from trauma or drowning.

The photographs illustrate the stomach tube and the various steps in its application on the rat.

THE GEIGER-MÜLLER COUNTER*

A Recent Instrument in Medicine and Medical Research

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A nontechnical description of a physical apparatus that has been finding applications in the field of medicine, especially in radiology, in radiobiology, and in physiology. It is the purpose of this article to describe the properties of the apparatus sufficiently well so that its function and some of its possibilities and limitations may be a matter of general record. A brief bibliography is included with references to significant historical and technical articles in the literature.

I. PROPERTIES AND USES OF THE GEIGER-MÜLLER COUNTER

WITHIN the past few years an instrument that had been primarily designed for physical studies of the atom (and more recently, for research in cosmic rays) has emerged from the physics laboratory to become a useful part of the general armamentarium of medicine. The instrument, known as the Geiger-Müller counter, is a physical apparatus for observing ionizing radiations of very low intensity.

The counter has an extreme sensitivity for this purpose since it is able to record the entry of a *single electron*, or the absorption of a *single photon* (light corpusele) within the limits of the sensitive chamber of the apparatus.

It is interesting to observe this unique property of the apparatus in comparison with even the most sensitive radiation detectors of more familiar usage (in various regions of the spectrum): thermopiles, photocells, air-ionization instruments, each of these being an apparatus which generally requires the incidence of many thousands of photons per unit area per second to produce an appreciable effect for observational purposes.

The counter is finding certain practical applications in medicine not only as a consequence of this property of extreme sensitivity, but also because of the fact that observations made with the instrument on ion-producing radiations of low intensity (and thus, for example, on very minute quantities of radioactive material) can be quite accurately made, and are consequently capable of quantitative interpretation.

In radiology the instrument has been used for detecting harmful stray radiations from x-ray and radium equipment. It is now the most practical device for finding lost radium,[8] and has already figured in many successful searches.† (Newspaper accounts of these interesting radium hunts refer to the apparatus as the "electric detective" or the "radium hen".)

*From the Prothero Radium Memorial, Chicago Memorial Hospital.
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†Dr. R. B. Taft, of Charleston, S. C., has prepared an interesting history of these stories of lost and found radium in book form: *Radium Lost and Found*, Charleston, S. C., 1938, J. J. Furlong & Son.

It is, however, in radiobiology and in physiology that the instrument may make contributions that are especially significant to medicine. During the past five years various means have been discovered for producing a temporary radioactivity in the atoms of many of the chemical elements (without changing their ordinary chemical behavior). These radioactivated elements and the Geiger counter have together opened a new avenue for tracing foodstuffs in the metabolic process,[1] or for observing the transfer of chemical substances during various physiologic activities.

The instrument has recently proved of great value in the quantitative physical detection of slight traces of radium in living victims of radium poisoning.[2] Although the total quantity of radium distributed throughout the body of an adult individual may be as little as two or three thousandths of a milligram, this amount can be determined with accuracy and with considerable speed by means of the Geiger counter.

II. THE INSTRUMENT IN RADIOLOGY; EXAMPLES OF ITS USE

An important problem to be met in x-ray and radium therapy institutions is that of protecting the personnel from harmful stray radiations. Several national radiotherapy and radiologic physics centers have provisionally accepted as "safe" those working conditions in which the intensity of the stray penetrating radiation is about 0.2 roentgens in eight hours or 4.2×10^{-4} (0.00042) roentgens per minute.* (For comparison: a total of 600 roentgens of filtered 200 kv. x-rays is necessary to produce a perceptible erythema on the human skin.)

We have built some Geiger-Müller instruments (Figs. 1 and 2) that have, among other things, served us in qualitative observations of stray radiation and radiation "leaks". The counter tubes of our apparatus have elements of such a size that, under test, each of the instruments was found to double its "natural count", of 10 impulses per minute, with a stray radiation intensity of about 1.7×10^{-7} (or 0.00000017) roentgens per minute.

This great apparent sensitivity is a real one in terms of roentgens. For clarity, however, it should be noted that the "roentgen," as a unit quantity of x-rays or of gamma rays, corresponds to an enormous number of photons: the individual unit particles of radiation. A directed beam of gamma rays having an intensity of one roentgen per minute, at a given point, sends about 2×10^9 or 2,000,000,000 gamma ray photons per minute perpendicularly through a one square centimeter area at that point.

The arbitrarily safe intensity of 4.2×10^{-4} roentgens per minute would ideally cause our own apparatus to respond with 25,000 impulses per minute. Such an intensity would be physically too great for our own rather simple apparatus to record quantitatively; paradoxically, we are able to make quantitative measurements only on radiations having $\frac{1}{250}$th to $\frac{1}{25,000}$th of the magnitude of this small intensity. (As shall be briefly explained later, Geiger-Müller counter instruments are usually built to suit certain ranges of radiation intensity, the tube element used being generally made smaller for greater intensities. There are circuits that permit counting up to 200,000 impulses per minute.)

It should be noted, by those using similar types of Geiger-Müller apparatus to observe the presence of stray radiations, that a large number of impulses may be registered under safe radiation conditions. The high sensitivity of such apparatuses may make it seem that

*This figure may be materially reduced because of recent considerations of the effects of even such low intensities of radiation in producing mutations in the germ plasm.

Fig. 1.—Counter built for operation on 110 volt AC line.

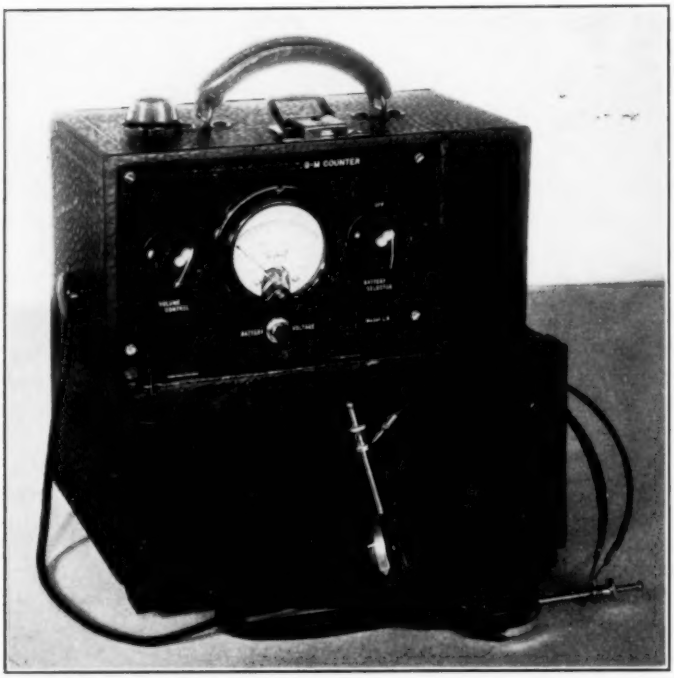

Fig. 2.—Battery operated counter instrument, independent of all external lines.

an alarming amount of radiation is present in a room, whereas (at an observed rate increase of 30 to 50 counts per minute) there may be only a few thousand photons passing through the counter tube each minute—a flux representing a truly safe amount of radiation in the room, as may easily be recognized by expressing this flow of photons in terms of fractional roentgens.

Lost radium is quickly traced with Geiger-Müller instruments, since the gamma radiation given off by the radium can be detected at an appreciable distance. Using the relatively small size element that is found convenient for our own apparatus, we get twice the natural count of about 10 impulses per

Fig. 3.—Battery operated instrument in use.

minute (due to intrinsic radioactivity of the surroundings and to cosmic rays), that is, twenty impulses per minute, with one milligram of radium at thirty feet free air-line distance.

Fig. 3 shows how the instrument is carried in a radium search (or in a stray radiation survey). We use the Geiger-Müller tube mounted within the case for mechanical and electrical protection. As viewed in Fig. 3, the tube* is mounted in the lower right hand corner of the case with its axis parallel to the lower leading edge.

*The tube, itself, and its properties are described in a following section.

III. G-M COUNTERS IN TOXICOLOGY: RADIUM POISONING, STUDIES OF LEAD POISONING

In biological investigations the counter apparatus is most generally used as a delicate assay instrument. With it one may measure quantities of certain radioactive substances that are present in amounts ordinarily determined only by microchemical methods. There are, indeed, instances where a microchemical method cannot be used, or is less sensitive than the measurement with the counter instrument.

One such example occurs in the diagnosis of radium poisoning. The quantity of radium element producing lethal effects in the human being is of the order of magnitude of one microgram. This small amount of radium may be distributed at random throughout the whole skeleton of a living individual. Under these difficult conditions it is nevertheless possible to detect chronic radium poisoning at any time by a physical procedure, whereas an interval of five to ten years can elapse before there may be an appearance of clinical symptoms. The detection, a quantitative one, is made by a relatively simple counter examination of the patient, through observation of the very weak gamma radiations emitted from his body.

Electroscopes, previously used for such work, are less sensitive than the counter, sometimes failing to detect, even qualitatively, the presence of an ultimately lethal amount of radium.

R. D. Evans[2, 3] and his colleagues have been chiefly responsible for developing a complete method: suitable counter and other important observation instruments, and an adequate technique, for the recent excellent quantitative physical studies made on radium poisoning. Evans[2] has devised a general method of determining the amount of a gamma ray emitting substance in a closed and inaccessible container. His method takes account of a random distribution of the radium in various bones and tissues, and of the internal absorption and scattering undergone by the gamma rays, so that a correct evaluation of gamma ray emitting substance within the body (radium C) may be made after a series of observations on the individual living person.

The complete physical study of radium poisoning of the individual involves other apparatus: gas collectors, sensitive ionization-electrometer instruments to record the amount of gamma radioactive principle (radium C) escaping in the exhaled breath as radon (a gaseous disintegration product of radium), and to observe the amount of radium being eliminated in the feces and urine.

(The total radium in the body is a quantity represented by the sum of the amount of radium C observed in the breath and of the radium C in the body itself. Radium, alone, is not appreciably gamma radioactive, it is the relatively short-life disintegration product radium C which emits most of the useful gamma rays.)

Using measurements made on several individuals, the data of which show a representative distribution of the radium in the skeleton, Evans has directly calibrated his counters for rapid measurement of other persons, and can make a fairly accurate survey of a single patient in from fifteen minutes to one hour with just the counter instrument.

Some of the results of this study prove quite interesting: observations show that 45 per cent of the radon produced by the radium in the body is exhaled with the breath; that cases of chronic radium poisoning normally eliminate 0.005 per cent of total body radium per day (as shown by earlier investigations); and that this small outgoing quantity distributes itself with considerable constancy in a percentage ratio of 91 per cent in the feces to 9 per cent in the urine. The work suggests the use of small traces of the radioactive lead isotope, radium D, as an indicator in a lead mixture for lead poisoning studies.

It is interesting to note that in 1923 Hevesy* had already used a radioactive lead isotope (thorium B) to trace the absorption and translocation of lead in plants, as a measure of lead metabolism, and also as a measure of the toxicity of lead. Hevesy ignited various portions of the plant and determined the active lead content of the ash concentrate with an electroscope. By adding radioactive isotope (to the inactive lead) in amounts that were increased in direct proportion to the extent of total dilution of lead in the nutrient medium, he was easily able to make measurements on very weak dilutions of lead, and thereafter to observe the disposition of a few hundredths or few thousandths of one per cent of the amount of lead initially made available to the plant.

Reeves and Morgan,† following a preliminary report by Taft,‡ have used the counter instrument to measure the retention of thorium dioxide (Thorotrast) within the human subject, after injection of the material for x-ray diagnostic purposes. (The weak gamma-ray emission of this material serves as an indicator of its presence, while the amount of radiation measured gives an approximation to the actual content of thorium dioxide within the subject.) Reeves and Morgan make no statement on the toxicity of the substance, but conclusively demonstrate its retention in large amounts even four years after the initial injection.

IV. USE OF THE G-M APPARATUS IN PHYSIOLOGY

From the statements of the foregoing section, it becomes apparent that the counter instrument forms one part of a dual team—of radioactive substance and sensitive detector—for investigating the fate of "tagged atoms" in physiologic processes. The English physiologist, A. V. Hill, has stated that artificially radioactive substances may have a meaning to present day biology much like that which the microscope had for an earlier era. Then the microscope revealed the cell; today, some aspects of the physiologic fate of the atoms themselves may be visualized with the new observational technique.

There are many examples available of the uses to which activated substances and counter instruments have already been put in physiologic investigations. The general procedure has been to "tag" the atom of a particular element within a compound; for example P, ordinary phosphorus (which is $_{15}P^{31}$§) in Na_3PO_4 is identified by intermixing with that compound an identical combination of a radioactive isotope of the element. (Example: $_{15}P^{32}$ in the $Na_3(_{15}P^{32})O_4$ molecule.)

*Hevesy, G.: Biochem. J. **17**: 439, 1923.

†Reeves, R. J., and Morgan, J. E.: The Retention of Thorium Dioxide by the Reticulo-Endothelial System, Radiology **29**: 612, 1937.

‡Taft, R. B.: The Radio-Activity of Thorium Dioxide Solution, J. A. M. A. **108**: 1779, 1937.

§15 is the atomic number, and 31 is the atomic weight.

Since the general chemical behavior of elements and their isotopes is identical in character, the labelled atom goes through the same molecular interchanges as its inactive brothers, and thus serves to reveal where they have gone. (It should be noted that this argument is true insofar as the radioactivity is so weak that no radiation changes are produced, for example in a colloidal system within which the radioactive substance may be.)

Simply considered, the basic process of making quantity observations with the counter is briefly this: the substance administered is measured under fixed conditions and rated at a given radioactivity factor, say, 1000. If, thereafter, some portion of the initially administered substance—suppose it be that contained within a complete thyroid gland—is found to have a radioactivity of 10, then one may say that 1 per cent of the material initially given found its way into the thyroid tissue.

Chiewitz and Hevesy[1] have made some studies on phosphorus metabolism in the rat, using radiophosphorus, with the counter apparatus as the assay instrument. The rats were fed a few milligrams of sodium phosphate containing ${}_{15}P^{32}$, and their separate organs and skeletal systems were later examined for radiophosphorus. These examinations showed that incoming phosphorus existed in certain characteristic ratios in the skeleton, in the spleen, kidneys, and brain. More particularly, in following the rate of elimination of ${}_{15}P^{32}$, the authors deduced that the average time which a phosphorus atom spends in the organism of a normally fed rat is about two months. They felt that their results lent more support to the view that the maintenance of bone structure is a dynamic process, the bone continually taking up phosphorus atoms, all or some of which are lost to be replaced again by incoming phosphorus. In examining different parts of the skeleton, Chiewitz and Hevesy found a conspicuous active phosphorus content in the teeth of the rat. The front teeth, which grow rapidly, contained a larger part of the incoming phosphorus, per gram, in a ratio of 10 to 1 (as compared with the whole skeleton) for an adult, and 6 to 1 for a young, growing animal. The molar teeth were found to take up less than the average amount of phosphorus per gram of basic weight, having a ratio as low as 1 to 2 compared with the skeleton.

In several investigations in plant physiology Hevesy and his associates* have performed some interesting and suggestive experiments that may readily find counterparts, as experiments, in animal physiology; for example, germinating maize and pea seeds were found to take up the labelled phosphorus in the germ but not in the endosperm, showing that there is no phosphorus exchange between the two.

Hamilton[7] has done some work which is useful in suggesting a method for studying the rate at which various elements pass from the digestive tract into the blood stream.

The present form of the experiment is quite interesting, though it is not truly quantitative in terms of the physiologic process involved: a subject receives, by mouth, a water solution of sodium chloride containing radiosodium (a short-life isotope of sodium which disintegrates with the ultimate emission

*Hevesy, G., Linderstrom-Lang, K., and Olsen, C.: Nature **137:** 66, 1936; **139:** 149, 1937. Hevesy, G., Linderstrom-Lang, K., and Nielsen, N.: Nature **140:** 725, 1937.

of gamma radiation). The subject holds in his hand a Geiger-Müller tube that is heavily shielded from the rest of his body by thick layers of lead. As the active radiosodium diffuses into the blood stream and passes through the vessels of the hand, the counter responds with an increasing barrage of impulses. A curve can be plotted showing a characteristic variation of the observed impulse rate with time, as increasing amounts of radiosodium reach the hand holding the counter tube. The exact shape of the curve is modified by several physiologic factors—which may be subject to inspection themselves, because of the modifications they produce.

V. HISTORY OF THE COUNTER; GENERAL PRINCIPLES OF ITS ACTION

While the counter has become available in a stable and rugged form only within the past two or three years, the essential operating principle of the instrument is not new.

In 1908 Rutherford and Geiger were making studies of the emission of alpha particles (positively charged helium nuclei) from the radium atom, and decided to use some device that would make a single one of these charged atomic particles detectable with the laboratory electrometer. They invented the novel principle of having the single charged particle initiate a sudden flow or discharge of a relatively large quantity of electricity between two electrodes in a rarefied gas chamber. This principle was based upon the known process of automatic increase of gaseous ionization by ionic-molecular "collision."

Alpha particles from a radioactive source were allowed to pass through a very thin aluminum foil "window" into a brass tube (see schematic drawing in Fig. 5*A*) containing gas at about one-eighteenth of normal atmospheric pressure. (The brass cylinder, itself [the cathode] and an inner central wire [the anode] served at the two electrodes, each being insulated from the other by ebonite plugs at the ends of the cylinder. The voltage between cylinder and wire was adjusted to a critical value just below that which would ordinarily produce a flashover, approximately 1300 volts in this case.)

As a single alpha particle sped past the window and then through the gas in the chamber, it left in its wake a large number of free ions split off from the neutral gas molecules. These nearly stationary ions in the wake of the fast particle were immediately swept up by the field and sent careering toward the electrodes, crashing into neutral molecules on their way and forming new ions by the same process with which they themselves had initially been released by the speeding alpha particle. The ionization was thus automatically and continuously increased until there were enough ions available to carry a surge of electricity through the circuit to the electrometer, which deflected electrostatically to announce the initial event: the entry of *one* charged atomic particle. (The whole process of the growth of the discharge in this way easily took place in less than one ten-thousandth of a second.)

In 1928 Geiger and Müller made an important addition to the principle of action of the apparatus by utilizing the metal wall of the chamber, now enclosed in a glass envelope, (see Fig. 5*B*) as the chief source of ionizing particles for the interelectrode gas volume. In this particular case the discharge was initiated by single electrons, either those originating uniquely from among the atoms of the cylinder material* or those that were released in and about the walls of the gas chamber by the absorption of photon radiation.

*As in the purposeful use of a cylinder made of pure potassium for the study of the very weak radioactivity of that metal.

The property of the tube of being sensitive to a single event of the absorption of electromagnetic (photon) radiation (an event followed by the release of a single free electron) made such instruments available for observing and measuring very weak electromagnetic radiations.

VI. COUNTER TUBES AND COUNTER CIRCUITS

Counter tubes are made of special forms for observing the various separate kinds of particle and electromagnetic radiations.

Electrically charged particle radiations,* when of low energy (low velocity and small penetrating power), can enter a counter tube only through a "window" sufficiently thin so that the particle will encounter relatively few atoms on its way into the chamber. Fig. 4*A* presents a schematic drawing of a tube

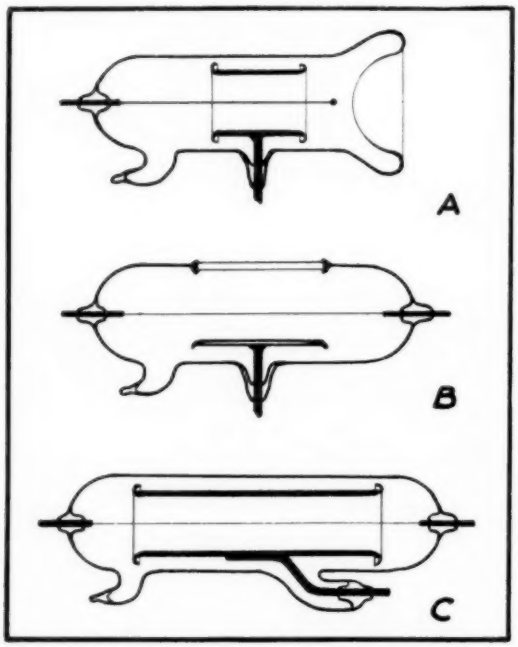

Fig. 4.—Types of counter tubes.

used in the observation of rather low speed electrically charged particles. The thin bubble of glass at the right provides a pressure barrier against atmospheric air and permits the moving electrical particle (coming as from a source on the right in the figure) to enter the low pressure gas chamber, there to initiate the ionization discharge mechanism.

**Alpha particles* (or alpha rays): individual helium atoms stripped of their two outer electrons, having a charge equal to + 2 electron units, and a mass equivalent to 4 units of atomic weight. (Ordinary hydrogen has an atomic weight of approximately 1, a unit that is the same as 1.65×10^{-24} or 0.000000000000000000000000165 gram.)

Beta particles (or beta rays): individual negative electrons of charge equal to - 1 electron unit. (For comparison: 6.3×10^{18} or 6,300,000,000,000,000,000 electrons passing a given point each second constitute a current of one ampere.)

The mass of the beta particle is 1/1840th of that of one unit of atomic weight.

Positrons: positive electrons, charge equal + 1 electron unit; mass equal to 1/1840th of that of one unit of atomic weight.

Protons (sometimes called "H particles," i.e., charged hydrogen particles): the nucleus of the usual hydrogen atom, charge equal to + 1 electron unit, mass equal to 1 atomic unit.

Deuterons: the nucleus of the double weight isotope of hydrogen, charge equal to + 1 electron unit, mass equal to 2 atomic units.

Photon or electromagnetic radiations (all of which travel with the speed of light, whatever their energy content) may enter a counter tube through the proper transparent aperture. Fig. 4*B* is a representation of the sort of tube used for very weak visible and ultraviolet light. It is essentially a gas-filled photoelectric cell and Geiger-Müller tube combined in one. A single photon may enter through the transparent plate, and, upon being absorbed by one atom of the cathode surface, causes the emission from there of a single photo-

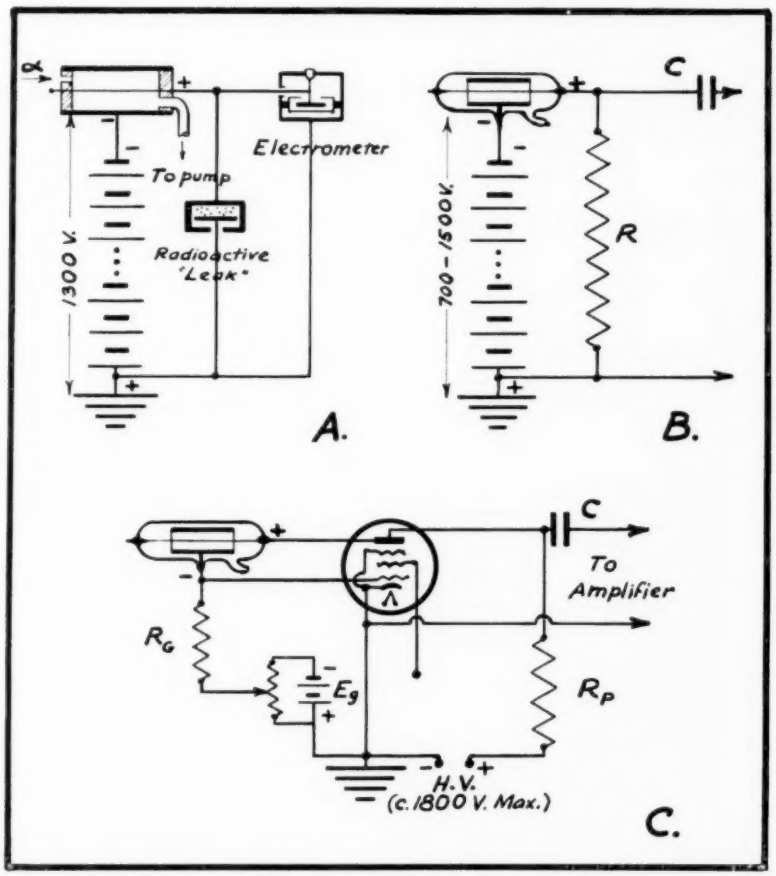

Fig. 5.—Types of counter circuits.

electron—a charged particle that is thereupon put to work initiating the trigger mechanism that announces the unique event.

Since photons of the familiar x-ray and gamma ray regions of the spectrum are quite penetrating, no special window or other aperture is necessary to admit the radiation to within the confines of the sensitive chamber of the tube. Fig. 4*C* shows the type of counter tube used for penetrating radiations. The tube element is made of some metal, such as copper or silver, which has a satisfactory absorption coefficient and adequate wall thickness for the radiation in question.

The tube intercepts radiation directed perpendicularly toward its axis in proportion to the longitudinal cross-section area of the metal cylinder element.

For intensities of gamma radiation, such as those from 1 to 10 mg. of radium at 20 to 30 feet, one might use elements of 2 to 3 cm. length and about 1 to 2 cm. diameter. For cosmic ray particles (which reach sea level at the rate of about one particle per 10 cm. square of horizontal area per second) one may have counter tube elements of as much as 50 cm. length (or greater), and 2 to 10 cm. diameter.

Three counter circuits are known in Figs. 5*A*, *B*, and *C*. These circuits, especially the last two, are of the type essential for the operation of the counter tube itself, and will be briefly explained. For the complete measuring instrument it is necessary to have the proper high voltage supply and proper impulse amplification and impulse recording circuits; these are mentioned in the last section.

Fig. 5*A* shows the initial circuit used by Rutherford and Geiger; it is now of historical interest, but the parallel between it and the succeeding types is quite distinct.

The circuit of Fig. 5*B* delivers pulses to the amplifier in the following manner: the flight of one ionizing particle through the sensitive volume of the counter tube (or the liberation of a single electron within its confines) immediately initiates the process of collision ionization. Within a time interval of the order of one hundred thousandth of a second the full discharge is formed, and a current flows around the circuit of voltage source, counter, and resistor (R). Passage of the minute current through the high ohmage resistor creates a gradient of voltage there (the IR drop) which polarizes the small condenser (C) and which then very shortly reaches a value sufficient to cut the potential across the counter tube down below that at which a discharge can be maintained. The discharge in the counter tube is thus "extinguished" by the current flow through the resistor. Thereafter the potential difference between the electrodes of the counter tube is restored while the charge flows off the small condenser toward the ground. During this interval of about one ten-thousandth to one-tenth of a second (depending upon the amount of capacity and resistance in the circuit) a "wave" of electrical polarization has passed over the plates of the small condenser and is impressed upon the amplifier circuit, which ultimately actuates a loud speaker in a sharp "click," flashes a neon tube, or drives an electrical or mechanical recording mechanism.

The circuit of Fig. 5*C* uses a vacuum tube to extinguish the discharge.[9] After the initial ionizing event, the current starting through the counter tube and R_G polarizes the control grid, G,* which releases a relatively large surge of electricity through the vacuum tube and resistor R_P; the current through the vacuum tube produces a large IR drop in R_P, and thus (in lowering the available voltage) effects rapid extinction of the discharge in the counter tube. Thereafter the charge on the coupling condenser (C) is permitted to flow to ground in a short time interval through the relatively small extinguishing resistor, R_P. The circuit is consequently restored to its initial sensitive state in a very brief interval of time.

*G must be maintained at a critical negative value by means of the small battery E_g for proper operation of the circuit.

VII. CRITERIA FOR THE USE OF THE APPARATUS

Geiger-Müller counters are logically useful only in those measurements or observations to which no other device can be better, or as well, adapted. There are many instances where the simple electroscope, or the electrometer, for example, is of equal merit with the counter or even more desirable (e.g., measurement of radium needles).

Quantitative chemical analyses of substances which are feebly radioactive can often be performed in the chemistry laboratory with the same facility, or even with greater speed and accuracy than is possible with an elaborate counter instrument. It is very difficult to make a casual, significant measurement when the radioactive principle is of short life, and when it is therefore necessary to make all of the measurements either under radioactive equilibrium or with each of the critical factors accounted for.

In order that there may be no duplication of effort in the biological aspects of work with the counter instrument, it is convenient to note that the physical radioactive characteristics of the naturally occurring radioactive substances are quite well known, and that quantitative information about them may be obtained from existing, complete tabulations.* Thus a chemical analysis can often be made and the total amount of radioactivity of a substance can be expressed in terms of known data.

One may designate two general uses for the counter instrument in biological work: (a) the simple, qualitative demonstration of the presence of low intensity radiation or of the presence of traces of radioactive substances; and (b) the quantitative measurement of minute amounts of radiation or of radioactive material in relation to biological systems, and also the exploration, thereby, of definite physiologic processes.

Instruments for qualitative observation should be free from any markedly erratic response. The sensitivity rating of such instruments, however, serves as no principal or definite measure of their accuracy.

Counter instruments for quantitative work (which are also the most efficient form for qualitative observations) are designed to embody certain very essential features for their proper function.

Since the response of the instrument to a given fixed radiation source varies with the voltage applied to the counter tube, it is necessary that the high voltage be kept quite constant, to within about one part in a thousand. Street and Johnson[11] and more recently Gingrich[5] have designed some simple and very effective voltage regulator circuits that are intended especially for use with counter instruments. These regulators maintain stability of the high voltage supply over quite a large variation of line voltages. Sets operating on 110 volt A.C. lines should have, beside these voltage regulators, adequate chokes and filters to suppress high frequency surges that may otherwise come through the lines and high voltage circuit up to the counter tube.

*Tables of constants of the radioactive elements are given in the following books:
Rutherford, E., Chadwick, J., and Ellis, C. D.: Radiations from Radioactive Substances, New York, 1930, Macmillan Company.
Kohlrausch, K. W. F.: Radioaktivität. From Wien and Harms: Handbuch der Experimental Physik, 1928.
Meyer, S., and Schweidler, E.: Radioaktivität, ed. 2, Berlin, 1927, Teubner.

The amplifier circuit should be able to receive each of the separate signals from the counter tube to the limit of the counting rate of the Geiger-Müller circuit itself. The amplifier should be so well shielded that it is insensitive to high frequency oscillations in its neighborhood.

Mechanical devices for recording the number of incoming pulses can be used at counting rates of the order 100 to 200 per minute. Near to and beyond this rate the inertia of most mechanical devices is sufficient to cause some loss of the count, or even to cause complete lack of response to pulses coming in at a very great rate. Electrical circuits have much less inertia than mechanical devices and can be used where it is necessary to count at very great speed. Gingrich, Evans, and Edgerton[6] described an arrangement which uses a meter deflection to give the average rate of arrival of the random counter pulses up to rates of several thousand per minute. Neher and Harper,[7] using a circuit due to Hunt,* and their own high speed Geiger-Müller circuit, are able to observe at rates up to 200,000 random pulses per minute. Thus it can be seen that Geiger-Müller circuits may be built with tubes and with recording systems adapted to widely different conditions of radiation intensity.

Within the counter tube itself and its immediate circuit, exist factors of central importance to the interpretation of the radiation measurement. Since every single photon and ionizing particle which traverses the sensitive volume does not initiate the discharge process, one speaks of the "efficiency" of the tube: the arbitrary ratio of the number of particles that are intercepted geometrically to the number that are physically effective in discharging the tube.

The efficiency of response of the counter instrument varies with the wave length of the different photons. For this reason the instrument is used essentially to compare the activity of two sources emitting radiations of very nearly the same spectral distribution, and not to make a direct, absolute measurement. Measurements of heterogenous stray radiations are thus "qualitative" unless they can be compared with a standard source having the same spectrum, or unless the spectral distribution is known and its effect on the counter can be deduced.

The numerical data given by the instrument—as in the regular comparison measurement—are significant and most nearly accurate when they have been corrected for two factors: (a) the finite operation time of the Geiger-Müller circuit (which involves a "counting loss" while the Geiger-Müller circuit is insensitive during the active discharge process), and (b) the statistical fluctuations due to the purely random incidence of the incoming particles. Locher and Weatherwax[8] show how such corrections may be made in a simple manner.

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TEST FOR OCCULT BLOOD*

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IN THE routine examination of specimens for occult blood with benzidine or orthotolidin it is always advisable to have a control in order to check the reagents, particularly in case of a negative reaction, because one cannot be sure the reagents are active unless they are checked.

In laboratories where blood is usually on hand it is a simple matter to add a drop of blood to the reagent and observe the reaction immediately. However, when a small laboratory or the general practitioner is making tests for occult blood, it is frequently found necessary to prick the finger in order to obtain blood. Furthermore, if the blood is not diluted sufficiently it does not indicate whether or not a trace of occult blood can be detected. It is necessary, therefore, to make a sufficiently high dilution of the blood before checking the reagent.

The following hemoglobin control solution has been prepared and used for the past year and a half. It is of a high dilution, has remained stable, conserves time, and prevents the danger of laboratory infection from frequent finger puncture.

The control reagent is prepared as follows:

To 20 c.c. of 50 per cent ethyl alcohol, 1.0 c.c. of defibrinated or oxalated blood is added.

Allow to precipitate for thirty minutes, stirring several times during this period.

Filter until clear, through Whatman No. 1 filter paper.

To the clear reddish filtrate, add two parts of distilled water.

A slightly opalescent, light amber solution results.

The reagent will keep indefinitely in a tightly stoppered bottle, a small dropper bottle will be found convenient for this purpose.

One or two drops of this solution will give a strongly positive reaction with benzidine or orthotolidin reagents.

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METHEMOGLOBIN DETERMINATION*

A Clinical Method

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METHEMOGLOBIN until recently has been a clinical oddity. Its formation as a complication of sulfanilamide therapy makes this nonfunctional derivative of hemoglobin assume wide interest. Speculation rather than analytical data, however, characterizes most statements in the current literature regarding the extent to which accumulation of methemoglobin accounts for the cyanosis and the loss of oxygen combining power of blood of patients receiving sulfanilamide.

The paucity of data on this important side reaction of sulfanilamide appears to be due largely to lack of simple methods for methemoglobin determination. Existing methods require expensive apparatus not found in most hospital laboratories (e.g., spectrophotometer, comparison spectroscope, bicolorimeter) or involve at least one determination of oxygen or carbon monoxide combining power. One relatively simple means of estimating methemoglobin would appear to devolve from determination of oxygen capacity by the Van Slyke method and determination of total pigment by one of the acid hematin methods (Newcomer, Sahli, etc.). If no abnormal pigment other than methemoglobin were present, the difference between total and active pigments should represent methemoglobin. The statement by Peters and Van Slyke,[1] however, that acid hematin methods do not give reliable total pigment values when applied to blood containing methemoglobin and the suggestion of Marshall and Walzl[2] that blood of patients receiving sulfanilamide contains aniline black must have deterred some from using this method. According to our experience, the Newcomer method gives reliable total pigment values on blood containing methemoglobin and we find no evidence that aniline black is present in the blood of sulfanilamide treated patients. Spectrophotometric measurements which will be published shortly indicate that, except in a small number of cases where sulfhemoglobin has been observed, methemoglobin is the only abnormal pigment likely to be encountered. We, therefore, consider the total pigment-oxygen capacity method suitable for clinical determination of methemoglobin.

We have examined several hundred specimens of blood from patients receiving sulfanilamide and have found methemoglobin in every specimen which contained more than 4 mg. per cent of sulfanilamide. Failure of others to detect methemoglobin is certainly due in some instances to use of the inadequate qualitative tests which are found in most textbooks of biochemistry and handbooks

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of clinical laboratory procedures. The statement so frequently encountered, that methemoglobin cannot be detected spectroscopically in blood unless it constitutes 20 to 30 per cent of the total pigment, is untrue. Methemoglobin can be detected spectroscopically in blood when it represents as little as 3 per cent of the total pigment.

A rapid and simple procedure for quantitative determination of methemoglobin is described below. The method requires special apparatus, but of an inexpensive nature. A determination can be carried out in less than five minutes if the fraction of blood pigment present as methemoglobin alone is desired. A knowledge of only the elements of spectroscopy is required of the analyst.

The determination depends upon the following facts: (a) Methemoglobin has a characteristic absorption band in the red portion of the visible spectrum at $\lambda = 630$ $\mu\mu$. (b) The width and the intensity of this absorption band are proportional to the concentration of methemoglobin. (c) Oxyhemoglobin, as compared with methemoglobin, absorbs little light in the red region of the spectrum.

In practice all of the pigment in one sample of a specimen of blood is converted into methemoglobin and the methemoglobin concentration of this is compared with that of an untreated sample of the same specimen. This comparison is accomplished by diluting the two samples until they have the same methemoglobin concentration, as determined by the width and the intensity of the characteristic absorption band. The ratio of these two dilutions represents the fraction of total pigment present in the form of methemoglobin.

APPARATUS

1. A Schmidt and Haensch hand spectroscope (*S* in Fig. 1) with adjustable slit and long focus magnifier is recommended because of its clear optical field and high dispersion.* This spectroscope has two moving parts, the telescope and the slit, the functions of which must be understood by the user. The telescope is for focussing on the spectrum and should be extended until the Fraunhofer lines (dark lines crossing the spectrum when the spectroscope is pointed toward the sky and the slit is nearly closed) are sharp. The slit determines the width of the pencil of light impinging upon the prism and thus the purity of the spectrum obtained. Maximum sensitivity in spectroscopic work is obtained when the slit is as nearly closed as is compatible with good visibility.

2. The specimen tubes (*ST.* and *U.*) are 100 c.c. graduated cylinders, the flared bottoms of which have been ground down parallel with the side walls. These should not be completely encircled by graduations, except perhaps at the 10 c.c. intervals, and should have the same diameter between the 5 and 15 c.c. calibration. This is checked by measuring the longitudinal distance between the 5 and 15 c.c. calibrations. Cylinders which differ by not more than 0.5 mm. are satisfactory.

*Hand spectroscope No. 225a and 227a Akatos, Inc., New York, N. Y. These two spectroscopes are identical except that 227a has accessories (a comparison prism and illumination mirror) which, although not required for methemoglobin determination, may be useful if the instrument is put to other use. See Harrison's *Methods in Clinical Medicine*, Macmillan, 1930, for brief discussion of the hand spectroscope.

3. The movable holder for the cylinders is a block of wood (dimensions, 3½ inches long, 2¼ inches wide, and 3¼ inches high) into or through which four holes are bored. The two vertical holes are for the cylinders and have a diameter ⅛ inch greater than the outside diameter of these. They are about 2 inches deep. Thin felt strips glued to the inner walls of these holes keep the cylinders in position when, in the course of comparing the spectra of the two solutions, the block is moved from side to side. The two horizontal holes (½ inch in diameter), which pass through the block at the level of the 15 c.c. calibration of the cylinders, permit a beam of light to pass through the solution in the cylinder and into the spectroscope. If the level of the solution in the cylinder does not stand above the beam of light, the cylinder is raised off the bottom and supported by a cork.

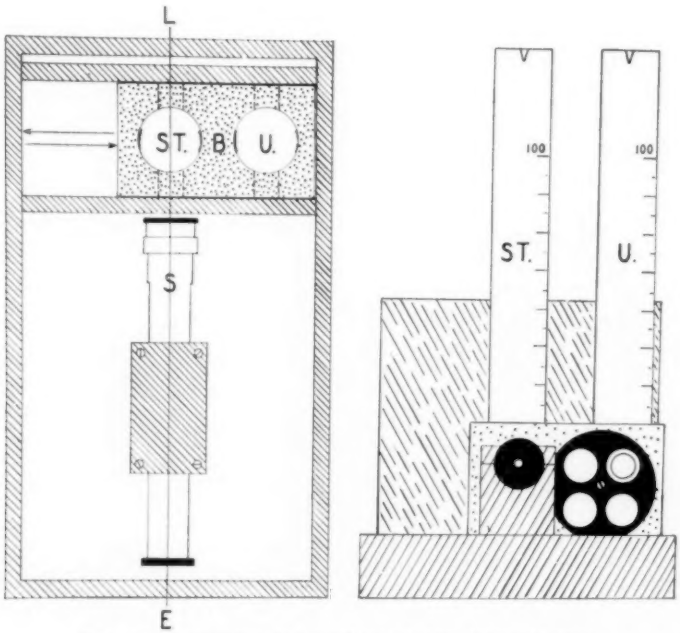

Fig. 1.—Apparatus for methemoglobin determination.

4. A red glass optical filter,* 3 mm. thick and about 20 mm. in diameter, is fixed on the cylinder block in front of the standard. A metal disk carrying three red filters, 3 mm., 2 mm., and 1 mm. thick, respectively, and having an additional hole not covered by a filter, is attached to the cylinder block before the unknown in such a manner that any one of the filters or no filter may be brought into the path of light passing through the solution. A single filter, 1 or 2 mm. thick, attached to the end of the spectroscope, can be used instead of the four, but with some sacrifice of convenience and accuracy.

5. The base of the apparatus, the holder for the spectroscope, the shield, and a cover (not shown in the illustration) are made of wood. A 60 watt bulb placed 2 or 3 inches behind a hole in the shield serves as a source of bright

*The Jena Colored Optical Glass Filter, RG-1, is recommended. It may be obtained from the Fish-Schurman Corporation, 230 East 45th Street, New York, N. Y., in 2 inch squares. The required sizes and thicknesses can be cut and ground from one such square of 3 mm. thickness.

light. The base shown in the illustration is attached to a second base upon which the light is fixed in order that the alignment of the spectroscope and the light will not be disturbed in the course of the determination.

REAGENTS

(1) 0.05 per cent Saponin solution. Use only a pure white powder which gives a perfectly clear solution. Preserve with toluene.

(2) 30 per cent Potassium ferricyanide solution.

(3) 20 per cent Ammonium acetate solution. Use only the reagent quality which has been kept tightly stoppered. This solution after diluting 1:20 should be pH 7.0 ± 0.2.

PROCEDURE

Measure 1 c.c. of blood into one of the cylinders. Lake for one minute with 4 or 5 c.c. of saponin solution and add 1 c.c. of the ammonium acetate solution.* Examine with the spectroscope for the presence of methemoglobin as follows: Starting with the slit of the spectroscope closed, gradually open it until red light is seen. If the blood contains methemoglobin in quantities greater than 3 to 5 per cent of the total pigment, a dark band will be seen across the red region of the spectrum. (This absorption band of methemoglobin disappears if the solution is made alkaline with sodium carbonate or sodium cyanide. The somewhat similar band of sulfhemoglobin is not so affected.) If the concentration of methemoglobin is very high, the field may be so dark that the methemoglobin band will not be visible until the blood is further diluted. If more than a trace of methemoglobin is present, prepare the standard methemoglobin solution as follows: Measure 0.5 c.c. to 1.0 c.c. of blood into another cylinder containing about 10 c.c. of saponin solution and after laking is complete (one minute), add 1 c.c. of ammonium acetate solution and 1 or 2 drops of potassium ferricyanide solution. Dilute further with saponin solution until the methemoglobin band is greatly decreased in intensity. An appropriate dilution of the standard is 1:100 if the total pigment concentration of the specimen is approximately normal. A proportionately smaller dilution is required with anemic bloods. The standard methemoglobin solution is now placed in the cylinder holder between the light and the spectroscope, and the slit of the spectroscope is adjusted until a moderately bright field is obtained. *Without changing the slit* the spectra of the two solutions are compared by rapidly moving the block from side to side. If the width and the intensity of the absorption bands of the standard and the unknown differ, the latter solution is diluted with saponin until the two are alike—or if the standard is stronger, it is diluted. The thickness of the filter used before the "unknown" should be increased as the dilution becomes greater. At low dilutions no filter is placed before the unknown. The thickness of the filter to be used at a given dilution is that which makes the orange-yellow portion of the spectrum between the methemoglobin band and the filter boundary alike as to width and intensity in the spectra of the two solutions. The dilu-

*The ammonium acetate provides a buffered solution, approximately pH 7.0. The buffer is required because methemoglobin is an acid-base indicator and because it is the spectrum of the acid form which is used in the determination. At pH 7.0 most of the methemoglobin is in the acid form and the oxygenation of the hemoglobin is not appreciably decreased.

tions at which the absorption bands of methemoglobin in the two solutions are alike as to width and intensity are noted. From these figures the fraction of pigment present in the blood in the form of methemoglobin is calculated. If absolute concentration of methemoglobin is desired, total pigment also is determined by a method which depends upon the formation of acid hematin (Newcomer, Sahli, etc.) or cyanmethemoglobin (Stadie, Wu) or by iron analysis (Kennedy, Wong). Colorimetric methods depending upon carbon monoxide (Palmer) and methods depending upon oxygen capacity (Van Slyke) cannot be used.

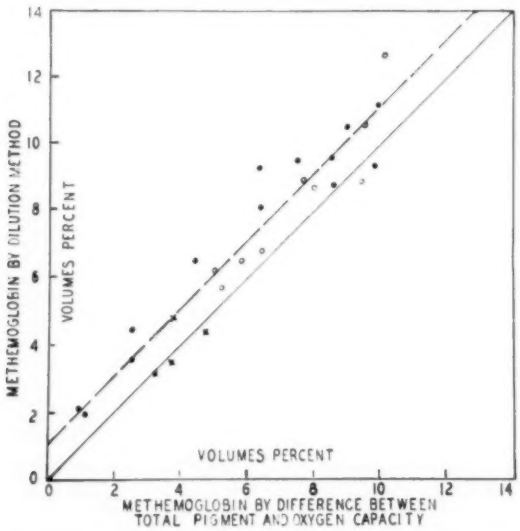

Fig. 2.—Comparison of results of methemoglobin determination by two methods. (The three different kinds of designations represent observations of three different individuals.)

CALCULATION

1. Percentage methemoglobin:

$$\frac{\text{Dilution of Unknown}}{\text{Dilution of Standard}} \times 100 = \text{Methemoglobin, as per cent of total pigment.}$$

2. Absolute methemoglobin concentration:

$$\frac{\text{Dilution of Unknown}}{\text{Dilution of Standard}} \times \text{Total Pigment} = \text{Methemoglobin in grams per 100 c.c. of blood, or volumes per cent, depending upon the choice of terms of expressing total pigment.}$$

Example: Standard, 0.5 c.c. of blood diluted to 40 c.c.; unknown, 1.0 c.c. of blood diluted to 20 c.c. Methemoglobin $= \frac{20}{80} \times 100$, or 25 per cent of the total pigment.

DISCUSSION

A comparison of the concentrations of methemoglobin in 26 samples of blood (from patients receiving sulfanilamide and dogs receiving nitrite) by the simple dilution method described here and the total pigment-oxygen capacity method shows remarkably good agreement. Total pigment was determined in most instances as acid hematin by the Newcomer method, in some as cyanmethemoglobin, spectrophotometrically. In Fig. 2 it is seen that the dilution method gives values for methemoglobin which are higher by about one volume per cent. This is true irrespective of the total pigment or methemoglobin concen-

tration. The broken diagonal line represents the mean deviation. The average deviation from this mean is only 0.6 volumes per cent. Most of these observations were made with a single filter of 3 mm. thickness and since it has been our experience that the subjective aspects of the method are improved by the use of filters of different thicknesses, it is probable that the method as described gives even better values. In any case it is adequate for clinical purposes.

Wu[3] and Stadie[4] state that acid hematin methods do not give reliable total pigment values when applied to blood containing methemoglobin. Both workers apparently used blood to which a large excess of ferricyanide had been added. Our experiments show that the Newcomer procedure gives correct total pigment values on bloods containing methemoglobin produced by nitrite.

SUMMARY

A simple, rapid, direct spectroscopic method for quantitative determination of methemoglobin in blood is described.

Addendum: A microadaptation of the above procedure, using one-fifth as much blood in standard and unknown and proportionately smaller amounts of the several reagents, and performing the dilutions in 10 c.c. instead of 100 c.c. graduated cylinders, permits determination of methemoglobin on finger-tip blood. For standard 0.1 c.c. of blood is diluted to 2 to 4 c.c., depending upon the total blood pigment concentration.

Further study of the method has shown that smaller hand spectroscopes than the ones recommended can be used without significant loss of accuracy.

The valuable assistance of Miss Jean Fischer is acknowledged.

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A NEW BABY BALANCE

ERICH LOEWENSTEIN, PH.D., FLUSHING, N. Y.

I WISH to describe a new baby balance which is used mostly in children's hospitals for the purpose of measuring the insensible perspiration. The insensible perspiration is an important physiologic function, providing one of the three avenues for loss of heat. When environmental conditions are reasonably constant, about 25 per cent of the total calories are lost by the evaporation of water from the body. Thus from the weight loss, the basal metabolic rate can be predicted. It has been shown by Levine and others that the prediction is sufficiently accurate for most clinical purposes. The method is particularly useful in the case of children, who, because of inability to cooperate, cannot be measured in the conventional basal metabolism apparatus. The sensitivity of the Sartorius baby balance has made it possible to introduce a damping device. Thus, since shorter periods can be used for weighings, a closer correlation can be maintained between loss of weight and extraneous factors which modify the metabolism. The balance can also be used for many other purposes, such as the calibration of volumetric flasks, where a high sensitivity is required with a large capacity. It can also be used for recording the growth of plants or animals, and for the insensible perspiration of animals. The accuracy of this balance is 10 mg. at a capacity of 25 kg.

The balance possesses several distinctive features: It is fitted with specially constructed suspensions (1) which compensate for the pendulum motion of the balance pans (2). This type of suspension compensates for oscillations in all directions.

It is equipped with circular arrestment (3). This arrestment disengages the three special steel knife edges (4) in such a way that friction across the knife edges cannot occur, a feature which results in an unaltered sensitivity for a long period of time. The arrestment may be equipped either with a wheel or, for smoother release, with a lever.

In place of the old type free swinging beam, the new balance has a simple oil-damping attachment (5) which brings the beam to rest after a single swing. A paddle is fixed to the beam in such a way that as the beam swings, the damping effect of the oil is brought to bear on the paddle. The use of a magnetic or electromagnetic type of damping was avoided since the accuracy of a balance can be affected by a magnetic field. The pointer (6) carries a photomicrographic scale (7), which by means of the projection device appears illuminated on a ground glass screen (8) against a hair line. The new projection device is fixed to the pillar to prevent zero point changes.

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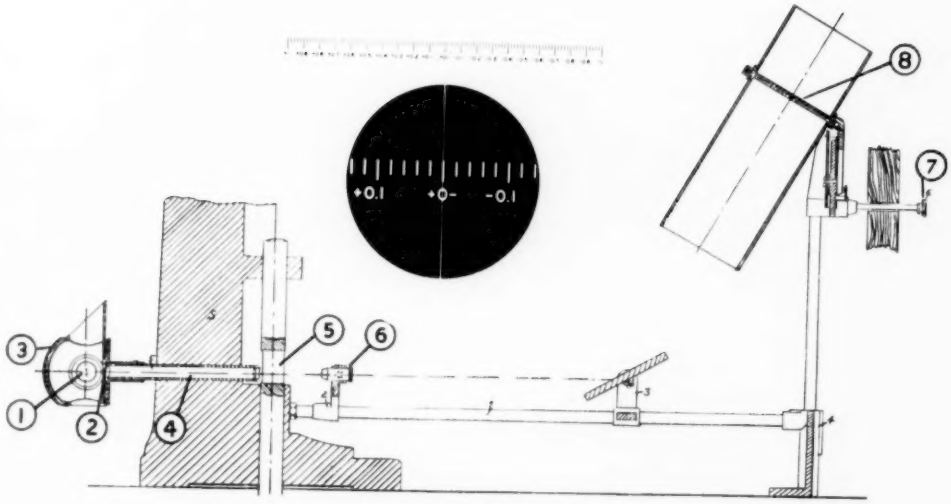

Fig. 1.

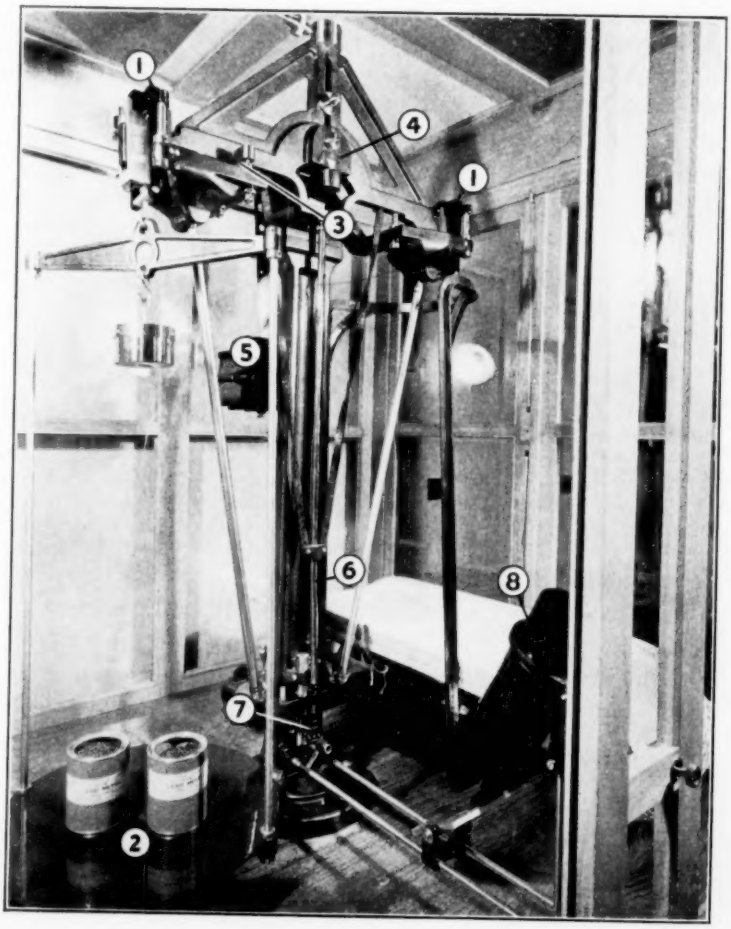

Fig. 2.

The new projection apparatus operates as follows:

An electric bulb (1) (see Fig. 2) of 10 to 12 volts mounted in a socket (2) serves as a source of light. The lamp housing (3) is covered entirely so that the infants will not be disturbed by the light. The electric current is drawn from the main through a transformer or resistance and thence to an automatic contact switch. This switch is operated by the knob for the combined pan and beam release. An alarm signal indicates that the arrestment of the balance has been released, and serves as a warning that the infant should not be removed at this time. Damage to the knife edges is thereby prevented. The light from the bulb is carried through a tube (4) which contains the condensing lenses. The light then passes through an optical scale (5) and through an objective (6). The objective permits adjustment of the sharpness of the image on the screen (8). A fine hair line permits adjustment of the zero point. This zero point adjustment is easily accomplished from outside the case by a screw (7). The device permits the reading of masses from 0.01 gm. to 1.0 gm. without the use of fractional weights. The scale is graduated on both sides into 50 divisions, each division being equal to 0.02 gm. By estimation between the divisions 0.01 gm. can easily be read. The figures read from 0.1 to 1.0 gm. and are designated as plus or minus, indicating that the scale reading is either to be added to, or subtracted from the final weight. The divisions and numbers on the scale appear sharply defined on a dark background and easily permit accurate readings from a distance. Therefore, a special table for the balance is not needed. These features allow one to make weighings with great rapidity. Errors associated with the handling and reading of weights and a rider, and from occluded dirt, etc., are entirely eliminated. The large projection of the scale on the screen permits accurate reading at a long distance without eyestrain.

The beam is of the short armed type (24 inches) and is protected with a black acid-proof varnish. The knife edges are of special hard steel, and the plates of fine agate. The diameter of the pan on the left side is about 15 inches. The pan holder is covered with acid-proof varnish, and has a height of 30 inches, and a width of about 15 inches. On the right side a special aluminum basket is mounted on the pan (length 40 inches, width 14 inches). This basket is covered with a net so that the infants do not come in contact with cold metal. Netting is also provided to prevent the babies from touching and disturbing the mechanism of the balance. The balance is completely enclosed in an oak housing with front and side doors. The dimensions of the housing are as follows: 56 inches height, 52 inches width, and 48 inches depth. The balance is mounted on a heavy oak base plate with leveling screws, and level on the base of the pillar.

To further simplify and speed up the weighing procedure, the balance can be equipped with a device for manipulation of weights from 1 to 100 gm. from outside the case. The device is operated by means of a double knob arrangement, consisting of an inner dial and outer ring. The total of these accessory weights is indicated by an arrow. By turning the inner dial, weights from 10 to 90 gm. can be added, and by turning the outer ring, weights from 1 to 9

gm. can be put on the auxiliary beam. The weights, which are placed on this notched beam over the left hanger, are horseshoe shaped and are suspended from long hooks which, when raised or lowered, put on or take off the weights. The weights so arranged will withstand severe jarring without falling from their suspension. This type of weight manipulator operates easily and eliminates much of the labor of adding single weights to the weight pan. The speed of manipulation of weights by turning the knobs is at least five times faster than handling individual weights with forceps. In addition, since the weights are not handled, they keep their accuracy, and are free from dust.

When weighings are to be made over a long period, say from one to two hours, it is perhaps advisable to provide curtains around the outside of the case so that the infant will not be disturbed.

In order to have reproducible conditions of temperature and humidity, a thermograph and hygrograph may be installed in the room. These instruments can be fitted with contacts which actuate relays, and operate an air-conditioning apparatus. The temperature of the baby can be measured by rectal thermocouple or by an electric resistance thermometer.

The results obtained with this balance are exceedingly satisfactory. The zero point has proved constant; the sensitivity is high; the damping device is effective; and the projection reading device is convenient. If for special purposes it is desirable to have a recording device instead of a projection reading device, a simple photomicrographic recording attachment can be furnished with the balance.

The author is deeply indebted to Pfaltz & Bauer, Inc., New York, who manufactured and installed this balance, for their invaluable aid in the development of this apparatus.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

STREPTOCOCCI, Hemolytic, Bacteriological and Clinical Study of Professional Personnel of Maternity Hospitals, With Special Reference to Carriers of, Bryce, L. M., and Tewsley, P. Med. J. Australia **1**: 639, 1938.

A brief description is given of an outbreak of a puerperal fever, involving three patients in a maternity hospital. The measures adopted to deal with it and those designed to prevent future similar outbreaks are outlined.

The value and limitations of periodic swabbing of the nose and throat of midwifery workers are discussed.

The bacteriologic technique is described. In addition to the recognized biochemical and serologic methods of classification of hemolytic streptococci, their sensitivity to four serologically distinct types of streptococcal bacteriophage was tested; it was not found to be in complete agreement with the results of the precipitin test. The variation of sensitivity to bacteriophage among strains falling into Lancefield's group A suggests, however, that this property might be used in epidemiologic studies.

The incidence of throat carriers of group A streptococci among the contacts of the three patients with puerperal fever was found to be 22 per cent. On two subsequent occasions, when there had not been recent known contact with streptococcal infections, the incidence of group A streptococci among similar groups of medical practitioners and nurses was 3 per cent and 6.7 per cent, respectively.

Cases are described and tables are given illustrative of the varying problems encountered in different individuals.

In persons with healthy respiratory tracts the presence of hemolytic streptococci in throat swabs appeared to be closely associated with recent contact with streptococcal illness. In those with clinically detectable abnormality of the nasopharynx there was a greater tendency to harbor hemolytic streptococci, irrespective of recent contact. It is, therefore, suggested that exclusion from midwifery units of persons with such abnormality, by means of preliminary medical examination, would be a possible way of reducing the risk of infection of patients and the disorganization attendant on their withdrawal during a period of midwifery work.

POSTMORTEM EXAMINATION of Stillborn and Newly Born Infants, Potter, E. L. Arch. Path. **25**: 608, 1938.

The post-mortem examination of the stillborn infant and the infant dying in the neonatal period presents certain differences from post-mortem examination of the adult. The causes of death in the stillborn and the liveborn infant are in general similar and are largely related to ante-partum and intra-partum obstetric complications. There is a small group of infants who succumb because abnormalities of the ova or spermatozoa have prevented normal development, and there is a small group who die from infections. Most of those remaining die from abnormal factors associated with the placenta, umbilical cord, birth processes, or the establishment of extrauterine life.

To determine the cause of death is frequently difficult, and if the body of the infant is the only material available, it is frequently impossible. To make it possible to arrive at a reliable conclusion, an adequate clinical history must be available, and the placenta, cord, and infant must be examined. Autopsy of the infant should invariably include examination of all parts of the body, including the cranial, thoracic, and abdominal cavities, the neck, the vertebral column, and the long bones. Microscopic examination of the viscera is essential.

JAUNDICE, Nature of the Bleeding in, Quick, A. J. J. A. M. A. 110: 1658, 1938.

Prothrombin deficiency is an important cause of defective and prolonged coagulation of the blood. A wide margin of safety, however, allows nearly 80 per cent of the prothrombin in human blood to be lost before serious hemorrhage occurs. In various types of jaundice the prothrombin may drop to an exceedingly low level, and it has been found that the bleeding tendency corresponds to the prothrombin depletion. Experimentally it has been demonstrated that the prothrombin can be reduced by deprivation of vitamin K, by a toxin such as occurs in spoiled sweet clover hay, and by an injury to the liver. In all types of prothrombin deficiency, blood transfusion is a prompt therapeutic means of stopping bleeding, since sufficient prothrombin is supplied to assure normal coagulation, but its beneficial action is temporary. The permanent cure depends on restoration of normal hepatic function. A vitamin K deficiency as a cause of hemorrhage has not been demonstrated clinically, but it is probable that it may be a cause of bleeding in man.

TISSUE: "Inflammatory Carcinoma" of the Breast, Taylor, G. W., and Meltzer, A. Am. J. Cancer 33: 33, 1938.

A clinical study is presented of 38 cases of inflammatory carcinoma of the breast seen at Pondville Hospital over a nine-and-one-half-year period.

This grave disease is not rare. The literature contains over a hundred cases. The incidence was 4 per cent of all breast cancers in our series.

Although the disease is rare after seventy, its age distribution is the same as for cancer of the breast in general.

The inflammatory signs may arise simultaneously with the cancer (primary type) or they may occur after a scirrhous cancer has been present for some time (secondary type). Pain is a common early symptom in the primary group. Inflammatory signs may result in mistakes in diagnosis and injudicious early therapy.

The primary cases come to medical attention early, yet their inflammatory signs are usually full-blown and the disease is widespread on admission. The cancer may have an acute erysipeloid distribution or show a tendency to nodular localization. Ulceration is rare.

The disease spreads rapidly in the superficial lymphatic structures of the chest wall. Multiple visceral metastases occur early, but the rapid course of the disease often does not permit them to attain clinical recognition. Bone metastases were recognized roentgenologically in only 4 primary cases. In the uncomplicated cases, leucocytosis, fever, and other signs of toxicity are rare. The patients maintain remarkably good health through the greater part of the course and cachexia is unusual. Death is most often due to intrathoracic complications. The average duration of life in primary cases was 21.3 months; in secondary cases 10.8 months after the appearance of inflammatory signs.

The inflammatory signs of edema, redness, and heat are due to extensive lymphatic blockage by cancer and congestion of the subpapillary plexus. There is no uniform pathologic type.

The large fatty breast seems a predisposing factor as does the hyperplastic breast of late pregnancy or lactation. No other predisposing factors could be established.

The results of therapy are poor. Surgery is followed by prompt evidence of supraclavicular disease, skin recurrence, or invasion of the opposite breast. X-ray seems to give the best palliative results. Artificial menopause does not alter the course of the disease.

GINGIVITIS, Acute Infectious, Black W. C. Am. J. Dis. Child. 56: 126, 1938.

The term "acute infectious gingivostomatitis" is proposed for that common but widely misunderstood and mistreated disease of the mouth in children which is characterized by marked gingivitis, oral fetor, fever, and enlargement of the regional lymph nodes. There are serious objections to the use of any one of the numerous terms now applied

to this disease. Its cause is unknown. It is unlikely that the oral anaerobic organisms, among which are Vincent's fusiform bacillus and spirillum, play more than a subordinate role in the pathogenesis. Oral anaerobic organisms of various sorts are constant inhabitants of the anaerobic crevices of the normal mouth, even in young children. They may temporarily increase in number during any gingival inflammation. Individually and collectively they are practically bereft of pathogenicity for laboratory animals.

By clinical and laboratory analysis in 69 cases, it has been found that acute infectious gingivostomatitis affects the hardy and well-kept child as commonly as it does the malnourished and dirty. It occurs most commonly in the fall and winter months, and the incidence is about twice as high in girls as in boys. The peak of age incidence comes at thirty months, while in four-fifths of all cases the disease occurs before the fourth birthday. Fever, the symptom most commonly present at onset, often precedes the appearance of local lesions by one to three days. The gingivitis and oral fetor frequently reach their peak of intensity after the constitutional symptoms have subsided. More than half of the patients have little round, shallow, gray oral or labial ulcers. Of the patients in which these ulcers do not occur, about five times as many are girls as boys, while the incidence of ulcers shows no significant sex difference. The disease, from the apearance of the first symptom to complete healing, usually lasts from nine to fifteen days.

The disease is benign and self limited. Recurrence and second attacks are rare. No important complications were encountered in the present series. The affliction is mildly contagious; the principal danger is that infection may occur in young children who come into intimate contact with a patient. Debilitated children or children suffering from measles should be rigidly protected from exposure to this disease.

RHEUMATIC FEVER, Serum Cholesterol in Patients With, Offenkrantz, F. M. Am. J. Dis. Child. 56: 68, 1938.

A complete series of 100 patients with rheumatic fever has been reported on. Forty-nine of these were taken as a control group, since they had no evidence of rheumatic activity. Evidence of such activity consisted of elevation of the leucocyte count above 10,000 per c.mm.; an erythrocyte sedimentation rate above 12 mm. per minute, and increasing cardiac damage, as shown by electrocardiographic study. The mean total value of the serum cholesterol (and standard deviation) was 181.9 ± 23.5 mg. per 100 c.c.; the mean value of the free serum cholesterol was 50.5 ± 7.4 mg.; the mean percentage of free cholesterol was 27.3 ± 2.23.

Of the remaining 51 patients, 5 died. Analyses of serum cholesterol within one day prior to or ten minutes after death revealed severe depletion of the cholesterol ester and only slight lowering of the free cholesterol level, so that the percentage of free cholesterol rose above 30 in each instance.

Eleven case histories were presented of children who seemed to suffer activity of the rheumatic fever throughout the period of observation. There was no cardiac failure of the right or left side, and all patients had negative Schick and Mantoux reactions. Here likewise the total cholesterol levels of the serum seemed to be definitely lowered and the percentage of free cholesterol elevated, so that the depletion was ascribed to the ester fraction.

In the next series (12 cases), the status of the disease fluctuated, periods of rheumatic inactivity being interrupted by flare-ups. The periods of activity again showed the lowered serum cholesterol level with the elevated percentage of free cholesterol (loss of ester). A general inverse relation was noted between the severity of the ester depletion (with a rise in the percentage of free cholesterol) and the sedimentation time, but this relation was not quantitative.

In 9 patients periods of cardiac decompensation were associated with the rheumatic infection. During such periods of failure, with passive congestion of the liver, the flare-up failed to produce rise in the percentage of free cholesterol, although there was a moderate

fall in the total cholesterol values. This indicated that both the ester and the free cholesterol content were lowered. A similar situation seemed to prevail in the 7 cases in which a fluctuating status of disease was complicated by a positive Mantoux reaction, though in 6 of these cases there was no clinical or roentgen evidence of active tuberculosis. Lastly, 5 cases were cited in which unusually high values for total cholesterol prevailed, despite activity of the disease. It was suggested at this point that possibly a psychiatric-functional status is associated with a particular type of cholesterol level in the blood.

The pertinent literature for this study is cited, and the salient findings are discussed.

BENZEDRINE, Color Reaction for, Richter, D. Brit. Lancet 1: 1275, 1938.

Using the micromethod described below, it was found that with doses of 20 mg. benzedrine sulfate in some subjects only 30 to 50 per cent is excreted in the urine in twenty-four hours, and benzedrine may still be detected in the urine forty hours after administration.

Benzedrine gives a yellow color with a number of nitrophenols, including picric acid, and this may be used as a convenient method of estimation for amounts down to 0.001 mg. per c.c.

To 60 c.c. urine in a 250 c.c. separating funnel, are added 6 c.c. petroleum ether and 4 c.c. 2 N caustic soda solution. The mixture is shaken three minutes by a rotating motion to prevent froth formation, allowed to stand two minutes, and the aqueous layer run off. The petroleum ether layer is centrifuged five minutes, cleared by stirring with a glass rod, and centrifuged for a further two minutes. Three cubic centimeters of the petroleum ether solution is measured in a dry pipette or 10 c.c. measuring cylinder and washed with 3 c.c. chloroform into a dry test tube; 0.6 c.c. of a solution containing 1 per cent picric acid in toluene is now added. The tube is allowed to stand well corked for twelve hours, during which time the picrates of other basic substances from the urine separate out; the clear yellow solution is then compared with a series of standards prepared in a similar manner from solutions containing 0, 1, 2, 5, 10, and 20 γ benzedrine per c.c. For making the standards pure liquid benzedrine was used and the solution titrated with standard N/5 acid, but for many purposes it is sufficiently accurate to use a solution obtained by heating a 10 mg. tablet of benzedrine sulfate with 7.35 c.c. water (the starch with which the tablets are made up does not interfere). The resulting solution contains 1 mg. base per c.c.

Normal urines often contain small amounts of amines which give a slight blank value corresponding to about 0.5 to 1.0 γ benzedrine per c.c. The value of the blank must, therefore, be determined and subtracted from the values obtained after giving benzedrine. Small amounts of indol in the urine do not interfere with the estimation, but the formation of amines by bacterial decomposition must be avoided by adding a few drops of toluene and using the urines as fresh as possible. Traces of moisture or alkali in the tubes interfere with the estimation, and it is essential that the tubes should be well cleaned with cleaning fluid and dried.

SPINAL FLUID, a Globulin Test for, Newman, K. O. Brit. Lancet 1: 1333, 1938.

The method suggested is considered superior to Pandy's and consists in using a 5 per cent solution of pure tannic acid in distilled water as a reagent, instead of phenol. The solution is rapidly made and keeps well. It is innocuous and can be prepared without laboratory equipment. The sensitivity of the test is very slightly greater than Pandy's, and the result of it can still be seen and estimated, after a considerable period, whereas the result of Pandy's test vanishes after a few minutes.

The test is best carried out by putting approximately 1 c.c. of 5 per cent tannic acid into a watch glass. One drop of cerebrospinal fluid is allowed to run into the solution, and the reaction reaches its maximum within a minute, after which it remains stationary. A positive reaction is graded in the usual way, as + or ++, or +++.

LIVER, Fatty Infiltration of, and the Development of Cirrhosis in Diabetes and Chronic Alcoholism, Connor, C. L. Am. J. Path. **14**: 347, 1938.

Fatty infiltration of the liver occurs in those conditions where, because of lack of intake or absorption of food, fat is mobilized from the existing fat depots; and where, because of internal interference with the metabolism of fat due to anoxemia or tissue anoxia, the accumulated fat cannot be broken down for use. In the first instance it results from external starvation; in the second, from what may be called internal or tissue starvation. In both instances normal carbohydrate-fat metabolism does not take place. Among the conditions in which this normal metabolism is altered or inhibited are the various diseases which, by their nature, are called wasting; the disease diabetes, and the disease following the introduction of poisons which inhibit proper tissue oxidation, the most common of which is alcohol. Other diseases occur but are not dealt with here.

In the starvation accompanying progressive morbid states the condition is of little pathologic importance, being in most cases terminal in nature. In diabetes the enlarged fatty liver may be influenced by insulin and by strict dieting, but also may persist for many years. In alcoholism a variety of factors operate to produce the same condition. Alcohol alone will cause some fatty infiltration, but as relative and sometimes absolute starvation is constantly associated with severe chronic alcoholism, the development of fatty infiltration of the liver most often depends on a combination of these two. The absence of vitamin B_1 in the diet may contribute also to this, but such deficiency is probably of minor importance.

Experiments cited in this paper, and analysis of existing recorded observations and experiments, indicate that a liver containing demonstrable neutral fat is in most cases pathologic; that fat may pass into and out of the liver with astonishing rapidity; and that fat may be present in such amounts as to interfere seriously with both the metabolic and the mechanical functions of the organ. In two cases of diabetes this chronic fatty infiltration went on to portal cirrhosis. One of these cases was so severe that the patient died of hemorrhage from esophageal varices. The development of perilobular fibrosis seems to be the result of a combination of mechanical pressure, local tissue anoxia, and general anoxemia, causing atrophy and degeneration of liver cells.

The alcoholic liver occurs in two forms, one of which is the precursor of the other. The first is the enlarged tense liver so swollen with fat that the distended surface lobules present the appearance of cirrhosis. This effect is further simulated by the intrahepatic block, which interferes with excretion of bile, and the transmission of portal blood. The clinical signs of jaundice and ascites are thus produced. Many such patients lapse into coma and die, and at autopsy the large liver is the only prominent finding. These show lobules distended with fat, and some show, in addition, hyaline degeneration and atrophy of peripheral cells associated with early proliferation of fibroblasts. A series of cases presented here represents this group, and another series illustrates the second form in which further proliferation of connective tissue produces the typical picture of portal cirrhosis as seen in chronic alcoholism. An unmistakable gradation of the one into the other is so manifest that the mechanism of the production of alcoholic cirrhosis seems to me to have been demonstrated. The absence of fat in many such livers at the end is explained by the exhaustion of body fat, by the discontinuance of alcohol consumption, and by the resumption of a normal or a high carbohydrate diet.